UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE VETERINARIA
Departamento de Nutrición, Bromatología y Tecnología de los Alimentos

AMINAS BIÓGENAS EN PRODUCTOS CÁRNICOS MÁS SALUDABLES EN BASE A SU CONTENIDO LIPÍDICO

MEMORIA PARA OPTAR AL GRADO DE DOCTOR
PRESENTADA POR

Mehdi Triki

Bajo la dirección de los doctores

Claudia Ruiz-Capillas Pérez
Ana María Herrero Herranz
Francisco Jiménez Colmenero

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TESIS DOCTORAL

Mehdi Triki

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Facultad de Veterinaria
Departamento de Nutrición, Bromatología
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Instituto de Ciencia y Tecnología
de Alimentos y Nutrición
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AMINAS BIÓGENAS EN PRODUCTOS CÁRNICOS MÁS SALUDABLES EN BASE A SU CONTENIDO LIPÍDICO

Memoria que presenta **Mehdi Triki** para optar al grado de Doctor por la Universidad Complutense de Madrid

Bajo la dirección y la tutoría de Dra. **Claudia Ruiz-Capillas Pérez,** Dra. **Ana María Herrero Herranz** y Dr. **Francisco Jiménez Colmenero**.

INSTITUTO DE CIENCIA Y TECNOLOGÍA DE ALIMENTOS Y NUTRICIÓN (ICTAN) - CSIC

Madrid, Junio 2013

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CERTIFICAN:

Que la presente memoria titulada "Aminas biógenas en productos cárnicos más saludables en base a su contenido lipídico", presentada por Mehdi Triki para optar al grado de Doctor, ha sido realizada en el Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC) bajo su dirección, y que, hallándose concluida, autorizan su presentación para que pueda ser juzgada por el tribunal correspondiente.

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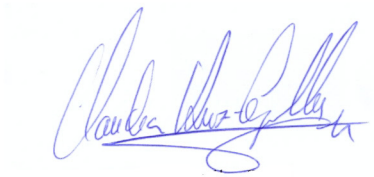

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To my mother, Nour of my life

& to my father's soul, you will always be in my heart…

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The True Legend

I've been searching for this true legend
I've been searching under seas
Down abysses, hunting as spy agent
I found the answer and release

I can't escape what I have already been.
I can't deny my dependence of it.
It is an object that everyone have seen
But never felt its magnitude and merit

I was about to catch it and go my way
Take it for myself and be selfish
But couldn't do it, since it has flown away
This treasure belonged to some kind of persons
Those were eternal, those never perish

Drifted by the wind through the destiny
Damned by the hope that's jailed on a cell
Slated by the trust through the hostility
Screwed by survival when it had to dwell

I was lost in my dreams
Lost between space and time
I knew I had to redeem
And follow my fitting rhyme

God, Houda & Hedi

God's faith was the key
Leading me to the right way
My parents made me see the tree
To the one that I most obey

Karim, Salma & Mariem

My path was full of risky ventures
I had to share with my own brother
He and my sisters were my mentors
With their help I had no kind of bother

Reaching the tree was a big challenge
I finally did it and was worth the bondage
There I met friends, the ones that derange
They couldn't let me take best advantage

Najm eddine, Besma, Jihene, Bilel, Nouha, Sonia, Cheker & Mariem

Thank God there were true mates
The ones that heal you from the dragons
By holding your hand on the debates
And jump with you into the wagons

Farah, Soumaya, Dalel, Moncef, Riadh, Nasr, Hamza, Anouar, Taha & Constantina

With their help I made the challenge
And won the pass to the upper level
I had to show a great form of courage
But with them it was worth the Travel

My grand parents, Niazi, Ines, Kamel, Elyess, Samar, Manel, my aunts, uncles & cousins

I've always been surrounded by love
Nourishing my soul, lightening my way
This thing has been sent right from above
Blessing my spirit and protecting my day

My love, Sana

Life didn't stop to make me realize
That it can be made like true paradise
When I gathered with love and was mesmerized
By a soul sent from heaven, made from sunrise

Family members of my love

I didn't gain only this angel
I gained a whole angelic family
They opened a path by lightening the candle
To a whole new life full of joy and sanity

To everyone (weather you are named during or before the poem)

Now I found the legend I was searching for
Thanks to God, life, love, hope and thee
I am proud to be part of your world
Because you, all, are now and forever part of me

Thank You

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RESUMEN / ABSTRACT

RESUMEN

INTRODUCCIÓN

Hoy en día, una de las principales tendencias que marca la evolución del consumo de productos cárnicos surge de la preocupación de los consumidores por la salud. De este modo se está incrementado el consumo de productos percibidos como más "saludables", los cuales para su desarrollo requieren procesos de reformulación encaminados a potenciar la presencia de compuestos beneficiosos para la salud, y/o limitar la de aquellos otros con efectos negativos, entre otros grasa, ácidos grasos saturados y sodio (Desmond, 2006; Jiménez-Colmenero et al., 2012)

En este sentido, la grasa es uno de los constituyentes de los alimentos a los que se ha prestado mayor atención debido a que es un factor que, a través de diversos mecanismos, condiciona en mayor o menor medida la aparición de diversos problemas de salud como enfermedades cardiovasculares, obesidad, cáncer, etc. En España en torno al 35% de la grasa ingerida diariamente (126 g) es de origen cárnico (Varela et al., 1996). Es por ello que una de las principales metas en relación con la salud radica en mejorar el contenido lipídico (reducir la proporción de grasa y aproximar su perfil de ácidos grasos a las recomendaciones de salud) (NAOS, 2005).

Por otro lado, el consumo de niveles elevados de sal (sodio) está directamente relacionado con un aumento de la hipertensión arterial que favorece la incidencia de enfermedades cardiovasculares. Dado que en España el consumo de sodio (9.8 g/día) es muy superior al recomendado (5 g/día) (NAOS, 2009) y que aproximadamente el 26% del sodio ingerido procede del consumo de derivados cárnicos, resulta esencial plantear estrategias de reducción de sodio en estos alimentos.

Sin embargo, hay que tener en cuenta que las estrategias, encaminadas a modificar la composición de los productos cárnicos, además de requerir cambios a nivel de reformulación, también pueden ir acompañados por modificaciones en los procesos de elaboración y conservación. Esto además de influir en las propiedades tecnológicas, sensoriales y microbiológicas de los productos, puede condicionar la formación de algunos compuestos potencialmente tóxicos para la salud, como por ejemplo las aminas biógenas (Ruiz-Capillas & Jiménez-Colmenero, 2004). Las aminas biógenas pueden causar migrañas, dolores de cabeza, problemas gástricos e intestinales, y respuestas pseudo-alérgicas, principalmente debidas a la acción tóxica de histamina y

tiramina. Además, algunos de estos compuestos (tiramina, putrescina y cadaverina) han sido señalados como precursores de nitrosaminas que son compuestos potencialmente cancerígenos. Estas aminas biógenas presentan además interés desde un punto de vista tecnológico ya que se pueden emplearse como índices de calidad en distintos productos sometidos a diferentes tratamientos.

OBJETIVOS

En base a estas consideraciones el objetivo de esta memoria ha consistido en desarrollar procesos de reformulación de derivados cárnicos encaminados a obtener productos más saludables, y estudiar cómo estos condicionan la formación de aminas biógenas. En tal sentido, se ha abordado el desarrollo de los procesos de reformulación dirigidos a mejorar el contenido lipídico (reduciendo la presencia de grasa y mejorando su perfil de ácidos grasos) y/o limitar la presencia de sodio en dos productos cárnicos con distintas características y condiciones de procesado: chorizo (producto crudo curado) y merguez (salchicha fresca originaria del norte de África). Todo ello encaminado a investigar cómo el efecto de estas estrategias de reformulación pueden condicionar la producción de aminas biógenas en las diferentes fases de procesado, maduración y conservación de los productos cárnicos modificados.

MATERIALES Y MÉTODOS

Optimización del método de determinación de aminas biógenas en productos cárnicos

Inicialmente la determinación cromatográfica por HPLC de aminas biógenas se basó en la metodología descrita por Ruiz-Capillas & Moral (2001). Se empleó para tal fin un equipo compuesto de una bomba cuaternaria (serie 200, Perkin Elmer, USA), un inyector automático (serie 200, Perkin Elmer, USA), un sistema de post-columna de Pickering PCX 3100 (Pickering Laboratories, Ca, USA) que contiene una columna de intercambio catiónico (K^+, 4 mm x 150 mm) y una pre-columna (K^+, 3 mm x 20 mm), ambas con un tamaño de partícula de 10 μm de diámetro (Pickering Laboratories, Ca, USA). El flujo de las fases móviles fue programado a 0,5 mL/min. La temperatura de la columna y de la pre-columna estaba programada a 40° C. La temperatura del coil de reacción fue de 45° C. El flujo del reactivo de post-columna (OPA) fue de 0,3 mL/min.

La detección se realizó utilizando un fluorímetro LC 240 (Perkin Elmer, USA) con una longitud de onda de excitación y emisión de 330 nm y 465 nm, respectivamente. El sistema estaba controlado mediante un integrador de datos PE Nelson (Perkin Elmer, USA). La adquisición de datos se realizó con el programa TotalChrom (Perkin Elmer, USA).

Las muestras de carne y productos cárnicos seleccionadas para el estudio de la optimización del método fueron carne fresca de cerdo (*Longuissimus dorsi*) y dos productos cárnicos: uno crudo curado "chorizo" y otro, salchicha tipo frankfurt, adquiridos en un mercado local.

Reformulación de productos cárnicos

Para la preparación de los diferentes productos cárnicos (chorizo y merguez) se empleó carne magra y tocino de cerdo en el caso del chorizo y carne magra y grasa de vacuno para el merguez. Los ingredientes no cárnicos empleados en la reformulación de merguez y chorizo fueron los comúnmente empleados en estos productos (pimienta, NaCl, sales de curado, trifosfato, comino, harissa, etc.). Los sustitutos de grasa estaban basados en el empleo de gel de konjac glucomanano con y sin aceite incorporado. La preparación del gel de konjac se realizo siguiendo la metodología de Jiménez-Colmenero et al. (2010a). Se elaboraron distintas matrices de konjac: konjac sin aceite, konjac con una combinación de aceites de oliva, lino y pescado y konjac con aceite de oliva.

Elaboración del chorizo

Los chorizos fueron diseñados y reformulados para reducir el contenido de grasa y/o mejorar el perfil de ácidos grasos con el fin de obtener diferentes niveles de grasa, utilizando la misma cantidad de carne magra, y por lo tanto de proteínas musculares. Los embutidos, de tamaño estándar (22/23 cm) se maduraron en las siguientes condiciones: 48 h a 23 °C y 90% de humedad relativa (RH), seguido de 13 °C, 70 a 80% RH, hasta el final del experimento. Cuando el experimento lo requería, los chorizos fueron envasados en bolsas de plástico y almacenadas en refrigeración (2 ± 2 °C) durante dos meses para su estudio.

Elaboración del merguez

Las salchichas frescas tipo merguez fueron diseñadas y formuladas para mejorar el contenido lipídico y reducir el nivel de sodio. Todos los productos contenían cantidades similares de carne magra de vacuno. La reducción del contenido de grasa se hizo sustituyendo la grasa animal con la misma proporción de dos análogos de grasas: gel de konjac y una mezcla de konjac con aceite de oliva.

En base a los resultados del primer estudio, en una selección de productos, se ensayó una estrategia de reducción de sodio basada en reemplazar el 50% del cloruro sódico añadido en la formulación inicial por una mezcla de sales que contenía 50% de KCl, $CaCl_2$ y $MgCl_2$. A fin de aumentar la vida útil de estos productos, se adicionó como conservante, metabisulfito de sódico, en los niveles marcados por la legislación (0,045%).

Caracterización de los productos

La viabilidad tecnológica, sensorial y microbiológica de los productos se evaluó a lo largo del procesado y durante la conservación en refrigeración.

Composición

Se realizaron los siguientes análisis: componentes mayoritarios, contenido calórico, perfil de ácidos grasos y minerales (AOAC, 2005; Serrano et al. 2005; Delgado-Pando et al., 2010).

Propiedades físico-químicas

Se determinó pH, perdidas de peso, perdidas por cocción, color, textura, oxidación lipídica, actividad de agua y contenido de nitritos y aminas biógenas (Delgado-Pando et al., 2010; Jiménez-Colmenero et al., 2010b).

Análisis microbiológico

Se llevaron a cabo recuentos de aerobios viables totales, bacterias ácido lácticas y enterobacterias, tanto en chorizo como en merguez

Análisis sensorial

Se realizó mediante escalas hedónicas por un panel de catadores semi-entrenados. Se evaluaron diferentes parámetros para cada producto.

Análisis estadístico

Se realizó empleando el SPSS Statistics 13.0, 14.0 y 17.0 (SPSS Inc, Chicago, Estados Unidos).

RESULTADOS Y DISCUSIÓN

Inicialmente, se consideró necesario desarrollar un método adecuado de determinación simultánea de distintas aminas biógenas en los productos cárnicos mejorando los procedimientos habitualmente utilizados (Henández-Jover et al., 1996; Ruiz-Capillas & Moral, 2001). La optimización de la metodología de determinación de aminas biógenas por HPLC ha permitido la cuantificación de nueve aminas biógenas (tiramina, histamina, β-feniletilamina, putrescina, cadaverina, triptamina, agmatina, espermidina y espermina) en diferentes matrices cárnicas (carne de cerdo, chorizo y salchichas tipo frankfurt) y condiciones de procesado. Las principales ventajas de este método optimizado fueron su versatilidad, sensibilidad y tiempo de ejecución, que se redujo con relación al método original (Ruiz-Capillas & Moral, 2001).

Una vez desarrollado el método de determinación de aminas biógenas se ensayaron procesos de reformulación en chorizo encaminados a mejorar su contenido lipídico y analizar como estos procesos condicionan la formación de aminas biógenas. La sustitución de grasa animal por un gel de konjac o por una matriz de konjac conteniendo una combinación de aceites (de origen vegetal y marino) resultó una estrategia adecuada para la obtención de chorizo potencialmente funcional en base a un contenido lipídico mejorado. Dicha estrategia, si bien permite reducciones importantes de grasa y dotar al producto de proporciones elevadas de ácidos grasos poliinsaturados, también presenta algunas implicaciones en las propiedades tecnológicas y sensoriales. En todo caso los productos obtenidos presentan niveles convenientes de aceptabilidad sensorial, sin consecuencias sobre el tipo y evolución de la microbiota. Sin embargo, dichos procesos de reformulación afectaron la formación de las aminas biógenas durante las etapas de elaboración y conservación de los chorizos. Durante el procesado, se apreció un aumento significativo de los niveles de las aminas biógenas más

representativas de la carne, afectando a los niveles de tiramina, putrescina y cadaverina en función del nivel de sustitución de la grasa animal. En general, a lo largo de la conservación se produjo un aumento en el contenido de las aminas biógenas, principalmente de tiramina, cadaverina putrescina y espermina, dependiente tanto del tipo de sustituto empleado, como de los niveles de sustitución de grasa realizados. Como ha sido señalado por otros autores (de las Rivas et al., 2008; Bover-Cid et al., 2009).

En una segunda etapa, se ensayaron las estrategias de reformulación de merguez encaminadas a mejorar su contenido lipídico. De igual modo se aplicaron procesos de reducción de sodio y aumento de vida útil por el empleo de un antimicrobiano como el metabisulfito sódico (SO_2). En todos los casos se valoró cómo estos cambios condicionaban la formación de aminas biógenas. La sustitución de grasa animal (vacuno) por un gel de konjac o por una matriz de konjac conteniendo aceite de oliva así como la sustitución de NaCl por una combinación de otras sales (KCl, $CaCl_2$ y $MgCl_2$), resultó una estrategia adecuada para obtener productos con reducciones importantes de grasa y sodio, presencia de ácidos grasos monoinsaturados, así como adecuadas propiedades tecnológicas y atributos sensoriales. Mientras que las modificaciones en el contenido lipídico no condicionaron la microbiota, la presencia del SO_2 originó una disminución acusada de la carga microbiana (independientemente de la reducción de sodio), y un aumento de la vida útil del producto. El efecto antimicrobiano en la producción de aminas biógenas fue similar a lo observado por otros autores (Bover-Cid et al., 2001; Bozkurt & Erkmen, 2002; Ruiz-Capillas & Jiménez-Colmenero, 2010). De igual modo se apreció un importante efecto en la reducción de aminas biógenas principalmente en tiramina, histamina y cadaverina, mientras que se produjo un leve aumento en los niveles de β-feniletilamina, putrescina y espermidina. En los productos elaborados sin el conservante, se observó un incremento muy significativo en los niveles de tiramina e histamina a lo largo de la conservación.

Con excepcion de las aminas fisiológicas, la formación de aminas biógenas en chorizo y merguez ha sido muy diferente. Tales diferencias (fundamentalmente en espermina, histamina y tiramina) cabe atribuirlas principalmente a los ingredientes/aditivos (tanto cárnicos, como no cárnicos) empleados en la elaboración de estos productos, algunos de los cuales condicionan el crecimiento de una flora

microbiana específica caso del SO_2. Otros como la harissa, pimentón, cilantro, hinojo etc., a los que también se les han atribuido propiedades antimicrobianos, pueden condicionar la microbiota en estos productos así como su capacidad aminoácido descarboxilasa (Tajkarimi et al. 2010).

En todo caso, los niveles de aminas biógenas encontrados tanto en el chorizo como en el caso del merguez pueden considerarse habituales en este tipo de productos y por debajo de los niveles que pueden suponer un factor de riesgo para la salud humana.

CONCLUSIÓN

Como conclusión general hay que señalar que a través de las estrategias de reformulación planteadas a lo largo de esta memoria, se pueden elaborar productos cárnicos saludables, de contenido en grasa y/o sodio reducido, estables, seguros, con propiedades tecnológicas y organolépticas adecuadas y con un perfil lipídico mejorado de acuerdo a recomendaciones nutricionales (menor cantidad de ácidos grasos saturados y mayor de poliinsaturados). Todo ello, hace que estos productos, chorizo y meguez, pueden ser sujetos de varias de las declaraciones nutricionales y de propiedades saludables de los alimentos.

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ABSTRACT

INTRODUCTION

Nowadays, one of the major trends which mark the evolution of meat products consumption has mainly emerged from consumers' concern for health. As a result, consumption of meat products, perceived as "healthier", is increasing. For the development of such products, reformulation processes are required in order to promote beneficial compounds presence, and/or limit those with negative effects such as saturated fatty acids and sodium (Desmond, 2006; Jiménez-Colmenero et al., 2012).

In this sense, fat is one of the foods constituents that have received greater attention since it is a factor which, through various mechanisms, conditions in greater or lesser extent the appearance of various health problems such as cardiovascular diseases, obesity, cancer, etc. In Spain, around 35% of daily ingested fat (126 g) is of animal meat origin (Varela et al., 1996). That's why one of the main goals in relation to health lies in improving lipid content (reducing fat proportion and approximate its fatty acid profile to health recommendations) (NAOS, 2005).

On the other hand, high levels of salt (sodium) intake are directly related to an increase in blood pressure that favors the incidence of cardiovascular diseases. Given that in Spain the sodium intake (9.8 g/day) is much higher than the recommended level (5 g/day) (NAOS, 2009) and that approximately 26% of the ingested sodium comes from meat products consumption, it is essential to propose strategies for reducing sodium in these foods.

However, it must be taken into account that strategies, aimed to modify meat products composition, besides requiring modification through reformulation, may also be accompanied by changes in elaboration and conservation processes. This, besides influencing technological, sensory and microbiological properties of the products, can condition the formation of some potentially toxic compounds for health, such as biogenic amines (Ruiz-Capillas & Jiménez-Colmenero, 2004).

Biogenic amines can cause migraines, headaches, gastric and intestinal problems, and pseudo allergic reactions, mainly due to histamine and tyramine toxic action. In addition, some of these compounds (tyramine, putrescine and cadaverine) have been identified as precursors of nitrosamines which are potentially carcinogenic compounds.

These biogenic amines also present interest from a technological point of view for its use as quality indexes in different products subjected to different treatments.

OBJETIVES

Based on these considerations, the objective of this thesis consisted in developing meat products reformulation processes aimed to obtain healthier products and study how these processes condition biogenic amine production. In this sense, the development of reformulation strategies has been approached aimed to improve lipid content (reducing fat presence and improving its fatty acid profile) and/or to limit sodium presence in two meat products with different characteristics and processing conditions: “chorizo” (dry fermented sausage) and merguez (North African fresh sausage). All of this is aimed to investigate how the effect of these reformulation strategies can conditions biogenic amines production in the different steps of processing, ripening and conservation of the modified meat products.

MATERIALS Y METHODS

Optimization of the method of biogenic amines determination in meat products

Initially, the chromatographic determination of biogenic amines by HPLC was based in the methodology of Ruiz-Capillas & Moral (2001). For this purpose a liquid chromatography, consisting of a quaternary pump (series 200, Perkin Elmer, SL Spain), an auto-sampler (series 200, Perkin Elmer, USA), a Pickering PCX 3100 post-column system (Pickering Laboratories, USA) containing a cation-exchange column (K+, 4mm x 150 mm) with a 10 μm particle diameter and a pre-column (K+, 3mm x 20 mm) also with a 10 μm diameter particle (Pickering Laboratories, USA) located in a Pickering PCX 3100 post-column system (Pickering Laboratories, CA, USA), was used. The mobile phase flow was programmed at 0.5 mL/min. The column and pre-column temperatures were programmed at 40 ºC. In the reaction chamber, the post-column reagent (OPA) flow was 0.3 mL/min. The temperature of the reaction chamber was kept at 45 ºC. Detection was done using an LC 240 fluorescence detector (Perkin Elmer, USA) at 330 nm excitation and 465 nm emission. All the chromatographic systems were controlled using a PE Nelson data integrator (Perkin Elmer, USA). Data

acquisition was carried out using TotalChrom software (Perkin Elmer Life and analytical Sciences, USA).

Meat and meat products samples, selected for the study of the optimization of the method, were fresh pork (*Longuissimus dorsi*), Spanish fermented sausages "chorizo" and frankfurter sausages which were purchased in a local market.

Meat products reformulation

For the preparation of the different meat products ("chorizo" and merguez), pork meat and fat were used for "chorizo" and beef meat and fat for merguez. Non-meat ingredients used in "chorizo" and merguez reformulation were the commonly used in these types of products (paprika, NaCl, curing salts, triphosphate, cumin, harissa, etc.). Fat substitutes were based on konjac glucomannane gel with and without incorporated oil. Konjac gel prepration was performed following the methodology of Jiménez-Colmenero et al. (2010a). Various matrices of konjac were elaborated: konjac without oil, with oil mixture combination containing olive oil, linseed oil and fish oil and konjac with olive oil.

Chorizo manufacture

Dry fermented sausages "chorizo" were designed and formulated to reduce fat content and/or improve fatty acid profile in order to obtain different fat levels using a similar amount of lean meat, and therefore of muscle protein. The sausages, of standard sizes (22–23 cm) were ripened under the following conditions: 48 h at 23 °C and 90% relative humidity (RH), followed by 13 °C, 70–80% RH, until the end of the experiment. When the experiment required it, the dry fermented sausages were packed in plastic bags and stored under refrigeration conditions (2 ± 2 °C) during two months for its study.

Merguez manufacture

Fresh merguez sausages were designed and formulated to improve fat content and reduce sodium level, using similar amounts of lean beef meat. Fat reduction was carried out by replacing the animal fat by the same proportion of two fat analogues: konjac gel and an olive oil-in-konjac matrix.

Based on the results of the first study, in a selection of products, a sodium reduction strategy was studied, based on the substitution of 50% of the added sodium chloride in the initial reformulation by a mixture of salts containing 50% of KCl, 28,58% of $CaCl_2$ and 21,42% of $MgCl_2$. In order to increase the shelf life of these products, a preservative, sodium metabisulphite, was added according to the levels set by the legislation (0.045%).

Characterization of the products

Technological, sensory and microbiological viability of the products was evaluated during processing and the refrigerated storage.

Composition

The following analyses were performed: proximate analysis, energy content, fatty acids profile and mineral contents (AOAC, 2005; Serrano et al. 2005; Delgado-Pando et al., 2010).

Physicochemical properties

pH, weight loss, cooking loss, color, texture, lipid oxidation, water activity and nitrites and biogenic amines content were determined (Delgado-Pando et al., 2010; Jiménez-Colmenero et al., 2010b).

Microbiological analysis

Total viable count, lactic acid bacteria and enterobacteria counts were performed in both “chorizo” and merguez.

Sensory analysis

The products were assessed by a panel through hedonic scales. The panelists evaluated different parameters for every formulation.

Statistical analysis

Statistical analysis were performed using SPSS Statistics software 13.0, 14.0 and 17.0 (SPSS Inc., Chicago, USA).

RESULTS Y DISCUSSION

Initially, it was necessary to employ a proper method of simultaneous biogenic amines determination in meat products by improving usually employed procedures (Henández-Jover et al., 1996; Ruiz-Capillas & Moral, 2001). The optimization of the biogenic amines determination methodology has allowed the quantification of nine biogenic amines (tyramine, histamine, β-phenylethylamine, putrescine, cadaverine, tryptamine, agmatine, spermidine and spermine) in different meat matrices (pork meat, dry fermented product and frankfurters sausages) and processing conditions. The main advantages of this optimized method were its versatility, sensitivity and elution time, which was reduced comparing with the original method (Ruiz-Capillas & Moral, 2001).

Once biogenic amines determination method was optimized, reformulation processes of dry fermented sausages “chorizo”, aimed to improve its lipid content and evaluate how this reformulation conditions biogenic amines production, were studied. Animal fat substitution by konjac gel or a konjac matrix containing a combination of oils (of plant and marine origin) resulted an appropriate strategy for obtaining potentially functional “chorizo” based on a lipid content improvement. This strategy allowed important fat reductions and provided the product with high polyunsaturated fatty acids proportions; also it presented some implications on the technological and sensory properties. In all cases, the obtained products presented suitable general sensory acceptability levels, without consequences on the type and evolution of the microbiological flora. However, these reformulation strategies affected biogenic amines formation during elaboration and conservation steps of “chorizo” sausages. During processing, the sausages showed a significant increase in the levels of the most representative biogenic amines in meat, affecting the levels of tyramine, putrescine and cadaverine, as a function of fat substitution levels. In general, during conservation, an increase in biogenic amines content was observed, mainly of tyramine, cadaverine, putrescine and spermine, depending on both type and level of fat substitution, as observed by other authors (de las Rivas et al., 2008; Bover-Cid et al., 2009).

In a second step, reformulation processes of fresh meat sausage merguez were studied aimed to improve its lipid content. Likewise, sodium reduction as well as shelf life increase, by using an antimicrobial agent as sodium metabisulphite (SO_2), was performed. In all cases, it has been evaluated how these changes conditioned biogenic

amines production. Animal fat (beef) replacement by konjac gel or a konjac matrix containing olive oil as well as NaCl substitution by a mixture of other salts (KCl, $CaCl_2$ and $MgCl_2$), resulted an appropriate strategy in order to obtain products with important fat and sodium reductions, monounsaturated fatty acids presence, as well as appropriate technological properties and sensory attributes. While lipid profile modifications did not condition the microbiological flora, the SO_2 presence originated a wide decrease of the microbial load (regardless of sodium reduction) and an increase in the shelf life of the product. The antimicrobial effect on the biogenic amines production was similar to the observed by other authors (Bover-Cid et al., 2001; Bozkurt & Erkmen, 2002; Ruiz-Capillas & Jiménez-Colmenero, 2010). Likewise an important biogenic amines reduction was observed, mainly of tyramine, histamine and cadaverine, while a slight increase in β-phenylethylamine, putrescine and spermidine levels was observed. In the products elaborated without the preservative, a very significant increase was observed in tyramine and histamine levels during the storage.

Except for the physiological amines, biogenic amines formation in "chorizo" and merguez was very different. These differences (mainly in spermine, histamine and tyramine) can be attributed mostly to the ingredients/additives (both of meat and non-meat origin) used in the manufacture of these products, some of which condition the growth of a specific microbial flora, SO_2 case. Other ingredients, such as harissa, paprika, coriander, fennel, etc., to which antimicrobial properties have been attributed, may condition the microbial flora in these products as well as their amino acid decarboxylase capacity (Tajkarimi et al. 2010).

In all cases, biogenic amines levels observed in both "chorizo" and merguez can be considered as regular in these types of products and they were below the levels that may result as a risk for human health.

CONCLUSION

As a general conclusion, the designed reformulation strategies used throughout this memory resulted in healthier meat products, elaborated with a reduced fat and/or sodium content, which were stables, secure, with appropriate technological and sensory properties and an improved lipid profile according to the nutritional recommendations (less saturated and higher polyunsaturated fatty acids amounts). All of this implies that

these products, “chorizo” and merguez, are likely to benefit from several nutrition and health food declarations.

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I. INTRODUCCIÓN

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I.1. AMINAS BIÓGENAS EN PRODUCTOS CÁRNICOS

I.1.1. Clasificación y formación de las aminas biógenas

Las aminas biógenas son compuestos nitrogenados no proteicos de bajo peso molecular que están naturalmente presentes en los organismos vivos y en los alimentos. Se clasifican según su estructura química en aminas aromáticas (histamina, tiramina, serotonina, β-feniletilamina y triptamina), diaminas alifáticas (putrescina y cadaverina) y poliaminas alifáticas (agmatina, espermidina y espermina) (Smith, 1980; Ruiz-Capillas & Jiménez-Colmenero, 2004). Estas aminas han sido también clasificadas dependiendo de su síntesis como "poliaminas naturales" y "aminas biógenas" (Bardócz, 1995; Ruiz-Capillas & Jiménez-Colmenero, 2004). Las poliaminas son aminas fisiológicas, naturalmente presentes en animales, vegetales y microorganismos (espermidina, espermina, putrescina y agmatina). Estos compuestos desempeñan un papel importante en la regulación de ácidos nucleicos y síntesis de proteínas, así como en la estabilización de las membranas celulares (Bardócz, 1995).

Las aminas biógenas propiamente dichas se forman por descarboxilación enzimática de los aminoácidos libres por acción de las enzimas aminoácido descarboxilasas, principalmente de origen microbiano (Figura I.1 y I.2).

I.1.2. Factores que influyen en la formación de aminas biógenas

El contenido de aminas biógenas puede variar dependiendo de distintos factores como, materias primas, microorganismos presentes y condiciones de procesado y conservación de los alimentos en general, y de los productos cárnicos en particular (Figura I.2).

I.1.2.1. Materia prima

La carne es la fuente natural de aminoácidos libres (AAL), y el medio donde se produce la reacción enzimática de descarboxilación que induce la formación de aminas biógenas. Cualquier condición que altere la naturaleza de la carne y sus características (nivel de AAL, contenido en grasa, pH, potencial redox, fuerza iónica, etc.) va a influir de una manera u otra en la formación de aminas biógenas (Figura I.2). Un mayor grado

de deterioro de la materia prima producirá un mayor contenido de aminoácidos libres. Sin embargo, la presencia de aminoácidos libres no es un factor limitante en la formación de aminas biógenas en productos proteicos como es la carne.

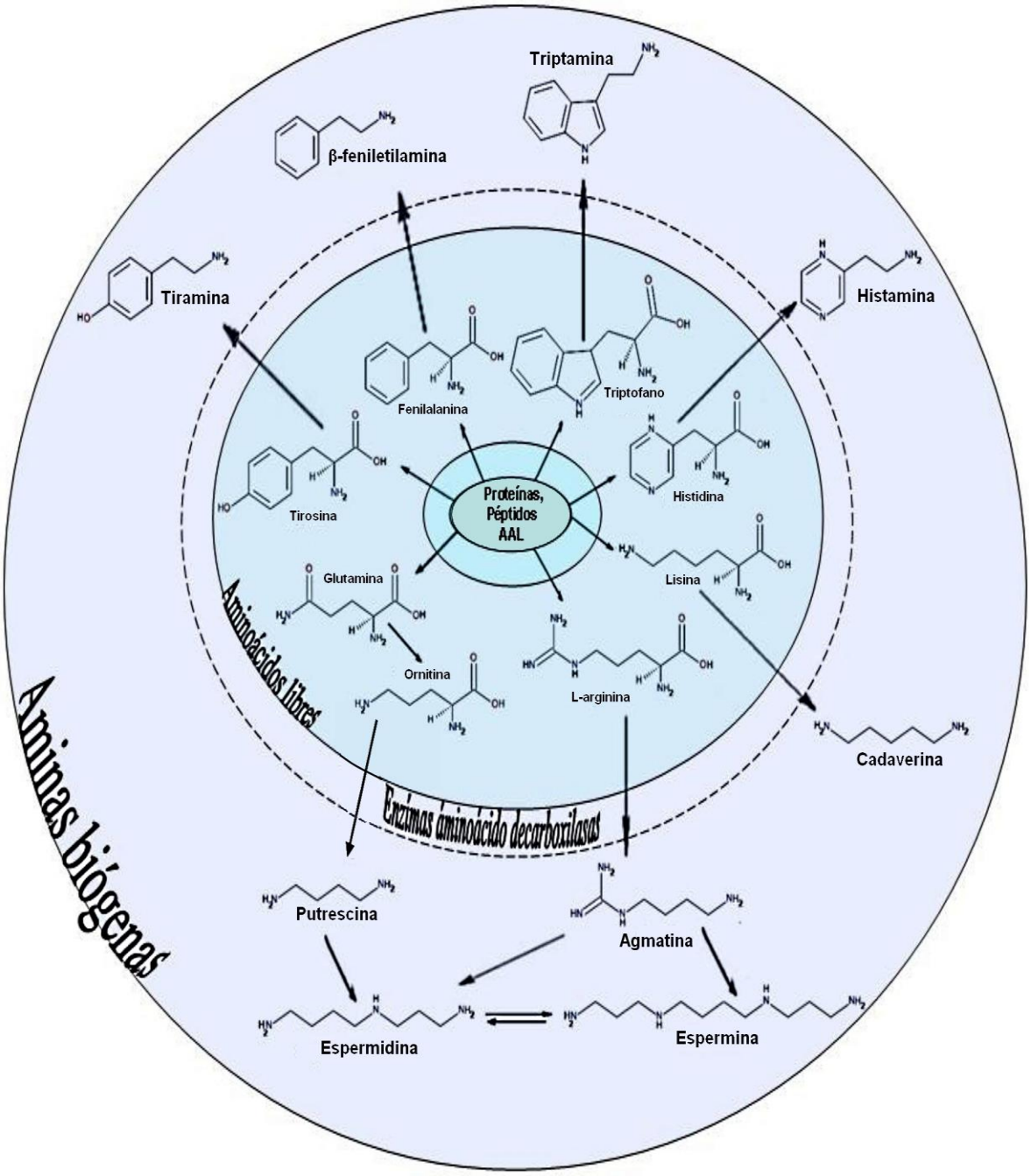

Figura I.1. Formación de las aminas biógenas a partir de aminoácidos libres.

Se ha demostrado que altos niveles de grasa disminuyen el contenido de aminas biógenas (Hernández-Jover et al., 1997; Kebary et al., 1999). Este fenómeno ha sido atribuido más a los cambios en la actividad de agua (a_w) que al contenido de grasa en sí. Una a_w baja produce una inhibición del crecimiento microbiano que se relaciona con un descenso en la formación de aminas biógenas.

Por otro lado, un pH ácido se ha relacionado con un incremento en los niveles de aminas biógenas. De hecho, a pH reducido las bacterias producen enzimas aminoácido descarboxilasa como parte de su mecanismo de defensa contra la acidez del medio formando aminas biógenas (Bover-Cid et al., 2006; EFSA, 2011). Por ejemplo, la actividad de la enzima histidina-descarboxilasa aumenta en medio ácido, con un rango de pH óptimo de 4 a 5,5 (Halász et al., 1994; Ruiz-Capillas & Jiménez-Colmenero, 2004). El pH final de la carne puede variar dependiendo de muchos factores que podrían influir considerablemente en la formación de aminas biógenas.

El potencial redox también se ha relacionado con la formación de aminas biógenas. Un potencial redox reducido estimula la producción de histamina, mientras que la histidina descarboxilasa parece inactiva en presencia de oxígeno (Karovičová & Kohajdová, 2005).

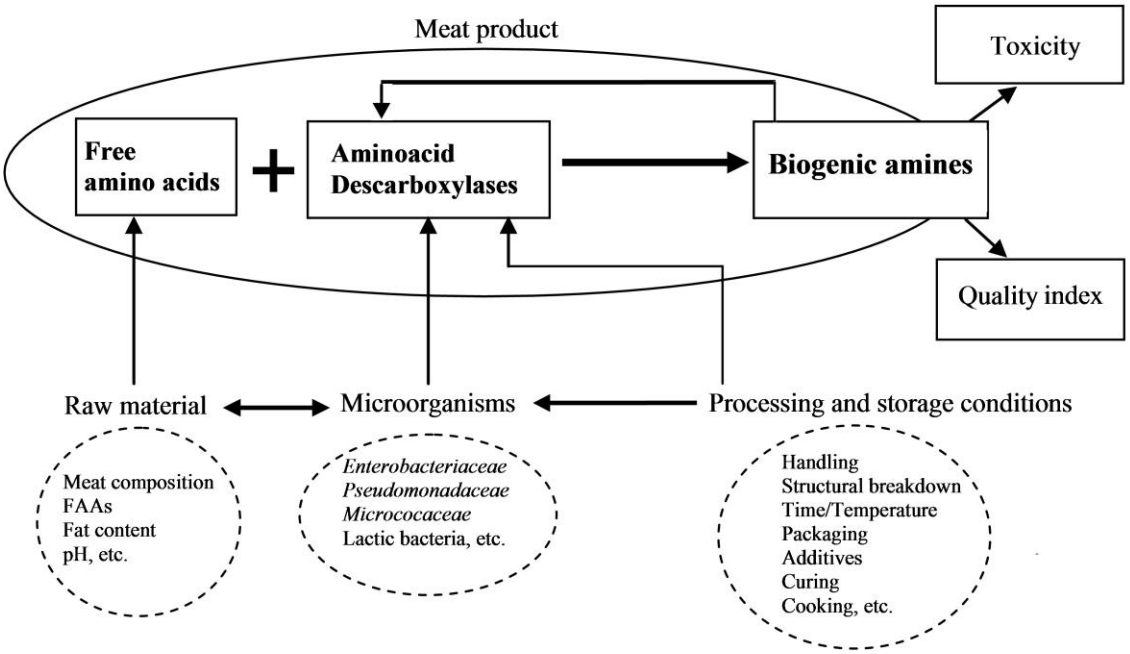

Figura I.2. Factores que influyen en la formación de las aminas biógenas (Ruiz-Capillas & Jiménez-Colmenero, 2004).

I.1.2.2. Microorganismos

Los microorganismos productores de las enzimas aminoácido descarboxilasa son otro de los factores fundamentales en la formación de aminas biógenas (Figura I.2). Los microorganismos principales productores de aminas biógenas (Figura I.2 y Tabla I.1) están en función del tipo de producto y las condiciones de procesado y conservación.

En general, la actividad aminoácido descarboxilasa en productos cárnicos es atribuible principalmente a *Enterobacteriaceae*, *Pseudomonadaceae*, bacterias ácido-lácticas (LAB) y *Micrococaceae*. Principalmente, a los géneros *Bacillus, Clostridium*, Pseudomonas, *Photobacterium, Staphylococcus, Micrococcus, Kocuria, Citrobacter, Klebsiella, Escherichia, Proteus, Salmonella* y *Shigella*. Además, muchas bacterias ácido-lácticas pertenecientes a los géneros *Lactobacillus, Enterococcus, Carnobacterium, Leuconostoc, Pediococcus* y *Lactococcus* también son capaces de descarboxilar uno o más aminoácidos libres (Marino et al., 2000; Suzzi & Gardini, 2003; Karovičová & Kohajdová, 2005; de las Rivas et al., 2008; Galgano et al., 2009). Se debe tener en cuenta que no se ha observado formación de aminas biógenas en carne estéril (Slemr & Beyermann, 1985; Ruiz-Capillas & Jiménez-Colmenero, 2004). Durante el deterioro de la carne fresca, las enterobacterias han sido identificadas como los principales productores de cadaverina y histamina (Ordoñez et al., 1991; Bover-Cid et al., 2001a; Bover-Cid et al., 2009) (Tabla I.1). Mientras que la putrescina ha sido asociada con altos recuentos de aerobios viables totales (TVC) y en particular de Pseudomonas (Edwards et al., 1983; Bauer et al., 1996) (Tabla I.1). En cuanto a la tiramina, se ha observado que sus principales productores son *Carnobacterium divergens, Lactobacillus curvatus, Lactobacillus plantarum, Bronchothrix thermosphacta*, Pseudomonas *y Escherichia coli* (Tabla I.1). Las bacterias ácido-lácticas son las principales formadoras de aminas biógenas en productos fermentados (Tabla I.1). De hecho, altos contenidos en *Lactobacillus* han sido asociados con la formación de elevadas concentraciones de aminas biógenas, principalmente de tiramina (Maijala & Eerola, 1993; Roig-Sagués & Eerola, 1997; de las Rivas et al., 2008).

Tabla I.1. Microorganismos productores de aminas biógenas en carne y productos cárnicos (adaptada de Ruiz-Capillas & Jiménez-Colmenero, 2004)

Products	Microorganisms	Biogenic amines	References
Fresh and cooked products			
beef, pork, lamb, poultry	*Carnobacterium divergens*	Tyramine	Leisner et al., 2007
Fresh beef	Pseudomonas	Putrescine	Edwards et al., 1987
	Bronchothrix thermosphacta, Pseudomonas	Putrescine Cadaverine Histamine Tyramine	Galgano et al., 2009
Fresh pork	Pseudomonas	Putrescine	Bauer et al., 1996
	Enterobacter cloacae	Putrescine	Halász et al., 1994
	Klebsiella pneumoniae	Cadaverine	
	Carnobacterium *Lactobacillus curvatus* *Lactobacillus plantarum*	Tyramine	Masson et al., 1996
Fresh Poultry	Pseudomonas	Putrescine Cadaverine	Rokka et al., 2004
Fresh lamb at 5ºC	*Enterobacteriaceae*	Cadaverine	Edwards et al., 1983
	Total viable count	Putrescine	
Wrapped and unwrapped fresh meat (pork, beef and rabbit)	*Enterobacteriaceae*	Cadaverine	Guerrero-legarreta & Chavez-Gallardo, 1991
	Pseudomonas	Putrescine	
Vacuum packed beef at 1ºC	*Lactobacillus divergens*	Tyramine	Edwards et al., 1987
	Hafnia alvey	Cadaverine	Dainty et al., 1987
	Serratia liquefaciens	Putrescine	
Fresh vacuum packaged beef	*Echerichia coli*	Putrescine, Cadaverine, Histamine Tyramine	Smith et al., 1993
Fresh pork stored in CO_2/Air and CO_2/O_2 (both at 20%/80%) at 2ºC	*Bronchothrix thermosphacta*	Cadaverine	Ordoñez et al., 1991
	Lactobacillus	Putrescine	
	Enterobacteriaceae		
Fresh pork sausage packaged in different atmosphere and under vacuum at 2ºC	*Shigella flexneri*	Putrescine Cadaverine Agmatine	Curiel et al., 2011
	Providencia vermicola	Putrescine Agmatine Tyramine	
	Aeromonas salmonicida	Putrescine	
	Carnobacterium divergens	Tyramine	
	Serratia grimesii, Serratia ficaria; Kluyvera intermedia; Enterobacter aerogenes; Yersinia kristensenii; Obesumbacterium proteus	Putrescine Cadaverine	
Smoked turkey breast fillets	Pseudomonas	Putrescine Cadaverine	Ntzimani et al., 2008
Fresh and Precooked chicken	Pseudomonas	Putrescine Cadaverine	Balamatsia et al., 2006; Patsias et al., 2006
Ground meat and processed meat products	*Escherichia coli*	Tyramine	Durlu-Özkaya et al., 2001
	Escherichia coli, Escherichia vulnaris, Escherichia fergusonii	Cadaverine	
	Escherichia coli, Morganella morganii, Proteus mirabilis	Histamine	
	Citrobacter freundii, Enterobacter, Serratia grimesii, Proteus alcalifaciens, Escherichia coli, Escherichia fergusonii, Morganella morganii, Proteus mirabilis, Proteus penneri, Hafnia, alvei	Putrescine	

Continuación Tabla I.1. Microorganismos productores de aminas biógenas en carne y productos cárnicos (adaptada de Ruiz-Capillas & Jiménez-Colmenero, 2004)

Products	**Microorganisms**	**Biogenic amines**	**References**
Dry, cured, ripened and fermented products			
Raw cured sausage	*Enterobacteriaceae*	Putrescine Cadaverine	Silla Santos, 1998; Bover-Cid et al., 2001a
Dry fermented sausage	*Staphyloccocus warneri, Staphyloccocus epidermidis*	Tyramine β-phenylethylamine	Martín et al., 2006
	Staphyloccocus xylosus	β-phenylethylamine	Tabanelli et al., 2012
	Lactobacillus curvatus, Enterococcus faecium, Enterococcus faecalis	Tyramine β-phenylethylamine	Bover-Cid et al., 2001a; Hugas et al., 2003; Aymerich et al., 2006; Latorre-Moratalla et al., 2010a
	Enterococcus faecium, Enterococcus faecalis	Tyramine	Pircher et al., 2007
	Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus casei/paracasei, Enterococcus faecium, Enterococcus faecalis	Tyramine Histamine	Komprda et al., 2010
	Lactobacillus curvatus Staphylococcus carnosus	Tyramine	de las Rivas et al., 2008; Komprda et al., 2009
Ripened sausages	*Enterococcus*	Tyramine	Roig-Sagués et al., 1997
	Lactic acid bacteria		
Turkish Soudjouck	*Enterobacteriaceae,* Lactic acid bacteria	Tyramine Putrescine	Ayhan et al., 1999
Botillo sausage	*Serratia liquefaciens, Enterobacter cloacae, Citrobacter braakii, Proteus vulgaris, Klebsiella terrigena, Rahnella aquatilis, Salmonella arizonae, Citrobacter youngae*	Tyramine Histamine Putrescine Cadaverine	Lorenzo et al., 2010
	Escherichia coli, Hafnia alvei	Tyramine Putrescine Cadaverine	
Fermented sausages	Pseudomonas, *Staphylococcus sciuri*	Putrescine Tyramine Histamine	Lu et al., 2010
	Lactobacillus farciminis	Tyramine	
	Enterococcus faecalis	Tyramine β-phenylethylamine	
	Enterobacter aerogenes	Putrescine Cadaverine Histamine	
	Enterobacteriaceae, Lactic acid bacteria	Putrescine	Bover-Cid et al., 2001a; Suzzi & Gardini, 2003
	Lactic acid bacteria	Tyramine	Bover-Cid et al., 2001a; Nowak & Czyzowska, 2011
	Enterobacteriaceae	Cadaverine	
Sucuk (fermented sausage)	Lactic acid bacteria	Tyramine	Gençcelep et al., 2007; Kurt & Zorba, 2010
	Enterobacteriaceae	Cadaverine	
Dry sausage	*Lactobacillus*	Histamine	Maijala & Eerola, 1993
	Carnobacterium, Lactobacillus curvatus, Lactobacillus plantarum	Tyramine	Masson et al., 1996
	Serratia	Putrescine Cadaverine	Bover-Cid et al., 2001a
Others			
Meat and meat products	*Bronchothrix thermosphacta*	Histamine Tyramine	Nowak & Czyzowska, 2011
Various meat products	*Enterobacteriaceae*	Cadaverine	Shalaby, 1996
	Pseudomonas	Putrescine	
	Streptococcus, Enterococcus faecalis, Coliforms, *Lactobacillus divergens*	Tyramine	
	Hafnia alvei, Klebsiella oxytoca, Morganella morganii, Edwardsiella, Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus divergens, Lactobacillus curvatus, Lactobacillus hilgardii	Histamine	

Sin embargo, se debe tener en cuenta que no sólo las concentraciones, sino también la especie y cepa de bacterias ácido-lácticas, podrían ayudar a explicar la alta variación en las concentraciones de aminas biógenas en este tipo de productos fermentados. De hecho, los principales productores de tiramina en productos fermentados son *Lactobacillus curvatus y Lactobacillus plantarum,* así como *Escherichia coli, Enterococcus faecium y Enterococcus faecalis*, mientras que la putrescina y cadaverina están producidas por las *Enterobacteriaceae* y la β-feniletilamina se origina principalmente por *Staphylococcus* y *Enterococcus* (Tabla I.1).

Por otro lado, se han descrito algunas cepas no formadoras de aminas biógenas como *Lactobacillus sakei*, *Pediococus pentosaceus* y *Staphylococcus xylosus* (Ruiz-Capillas & Jiménez-Colmenero, 2004). Este hecho es importante tenerlo en cuenta a la hora de diseñar cultivos iniciadores, lo que podría prevenir la formación de estas aminas biógenas en embutidos fermentados (Maijala et al., 1995; Bover-Cid et al., 2001a). Sin embargo, cualquier reducción siempre dependerá, no solo del cultivo iniciador, sino además, de otros factores que influyen en la formación de aminas biógenas, especialmente la materia prima.

I.1.2.3. Reformulación, procesado y conservación

Los procesos de reformulación, procesado y conservación de los productos cárnicos son también factores importantes que influyen en la formación de las aminas biógenas (Figura I.2).

Hoy en día, **la reformulación** de los productos cárnicos tradicionales es una práctica muy empleada por la industria para mejorar el perfil nutricional de los mismos (Jiménez-Colmenero et al., 2012). La reformulación incluye entre otras estrategias la reducción de ciertos ingredientes perjudiciales para la salud como la grasa y el sodio, así como cambios en el uso de aditivos que tienen efectos antioxidantes y antimicrobianos (nitratos y nitritos, sulfitos, etc.) (Jiménez-Colmenero, 2005).

La **reducción de grasa** mediante el empleo de distintos análogos de grasa animal puede tener efectos diversos sobre la producción de aminas biógenas dependiendo del tipo y nivel de sustitución así como del tipo de producto cárnico. Así, se ha observado que la sustitución parcial y total de la grasa animal por un polisacárido como el konjac glucomanano en un producto cárnico cocido como el pâté no presentaba un efecto claro sobre la producción de aminas biógenas (Delgado-Pando et al., 2012). Resultados

similares han sido observados en relación con la sustitución parcial y total de la grasa animal por una emulsión conteniendo una mezcla de aceites ricos en ácidos grasos poliinsaturados *n-3*. El uso de este tipo de emulsión como sustituto de grasa en salchichas tipo frankfurt, tampoco originó efectos en la producción de aminas biógenas (Delgado-pando et al., 2011a). Sin embargo, la adición de nueces en un reestructurado cárnico fresco elaborado con transglutaminasa favorecía la producción de tiramina, histamina, putrescina y cadaverina, mientras que la agmatina no experimentó cambios en comparación con la muestra control (Ruiz-Capillas et al., 2004). También en reestructurados de pollo elaborados con algas (*Himanthalia elongata*) y transglutaminasa, se observó un aumento de los niveles de β-feniletilamina y putrescina en comparación con el control (Cofrades et al., 2011).

La sal (NaCl) empleada en la formulación de productos cárnicos también se ha relacionado con la producción de aminas biógenas, principalmente a través de su papel en la reducción de la actividad de agua (a_w). Se ha observado que 30 g de NaCl /kg en productos fermentados aumentan la producción de tiramina favoreciendo el crecimiento de *Lactobacillus curvatus* (Straub et al., 1994). Sin embargo, la presencia de niveles bajos de sal en reestructurados de pollo se ha relacionado con un aumento en los niveles de tiramina (Cofrades et al., 2011). Karovičová & Kohajdová (2005) observaron que el cloruro sódico activa la enzima tirosina descarboxilasa e inhibe la actividad de histidina descarboxilasa. Sin embargo, varios autores (Suzzi & Gardini, 2003; Roseiro et al., 2006) observaron que el contenido de diversas aminas biógenas (cadaverina, putrescina, tiramina y β-feniletilamina) disminuía notablemente con el aumento de la concentración de NaCl. Por tanto, parece lógico suponer que el efecto del NaCl está relacionado no solo con su concentración sino también va a depender del tipo de cepa.

Los nitratos y nitritos, muy utilizados en la elaboración de productos cárnicos, influyen también en la producción de aminas biógenas. Varios autores han observado que su presencia reduce la producción de aminas biógenas en productos cárnicos fermentados como el sucuk (Bozkurt & Erkmen, 2002; Kurt & Zorba, 2010). Se ha observado una disminución en los niveles de la cadaverina y tiramina en sucuk elaborado con mayor nivel de nitritos (Kurt & Zorba, 2010).

Antimicrobianos como los sulfitos son empleados comúnmente en la elaboración de ciertos productos cárnicos. El sulfito sódico es un antimicrobiano que reduce principalmente la proliferación de mohos, levaduras, bacterias gram negativas y *Lactobacillus*. Además, de su efecto antimicrobiano, también se le han atribuido

propiedades antioxidantes que retrasan las reacciones de decoloración del producto cárnico. En productos curados, este antimicrobiano inhibe la producción de aminas biógenas y especialmente de cadaverina mientras que promueve la producción de tiramina y putrescina (Bover-Cid et al., 2001b). Otros antimicrobianos como el sorbato de potasio y/o sodio también son inhibidores de la flora microbiana y suelen ser utilizados para limitar la formación de aminas biógenas en los productos cárnicos (Shalaby, 1996). La adición de glucono delta-lactone (GDL) en salchichas fermentadas también reduce los niveles de histamina y putrescina (Maijala et al., 1993).

Ingredientes de origen vegetal como el cúrcuma, pimentón, pimienta, jengibre, ajo, cebolla, clavo, canela, etc. tienen también un efecto inhibidor de la producción de aminas biógenas en distintos tipos de alimentos (Shakila et al., 1996; Mah et al., 2009).

Por otro lado, la producción de aminas biógenas esta condicionada por el tipo de producto cárnico. De hecho algunos autores (Wortberg & Woller, 1982; Vidal-Carou et al., 1990) han observado que la formación de histamina y tiramina es menor en productos cárnicos elaborados solamente con una pieza de carne de cerdo (por ejemplo jamón cocido) en comparación con otros preparados con una mezcla de diferentes partes y grados de desintegración estructural (salami, salchichón, chorizo o salchichas de Bolonia), donde se producía mayor manipulación y mayor tendencia a la formación de aminas biógenas.

La carne y productos cárnicos sometidos **a <u>tratamientos térmicos</u>** presentan niveles inferiores (entre 4 y 10 veces) de aminas biógenas que los productos fermentados (Tabla I.2). Altas temperaturas reducen la carga microbiana y con ello la formación de aminas biógenas. La existencia de aminas biógenas en productos cárnicos cocidos se debe principalmente a su presencia en la materia prima utilizada y las condiciones de manipulación durante la elaboración (Ruiz-Capillas & Jiménez-Colmenero, 2004). Además, se debe tener en cuenta que factores asociados al tratamiento térmico (temperatura, velocidad de calentamiento, etc.) también influyen sobre la enzima aminoácido descarboxilasa, que en la mayoría de los casos se vuelve inactiva a temperaturas de 65 ºC (Maijala et al., 1995; Kebary et al., 1999). Así en productos cocidos como jamón cocido, salchichas tipo frankfurt, pâté, etc., se han observado reducciones significativas en los niveles de aminas biógenas como tiramina, histamina, putrescina, cadaverina y β-feniletilamina (Tabla I.2). Por contra, no se han observado diferencias en los niveles de las aminas fisiológicas (espermidina y

espermina), los cuales dependen principalmente de la naturaleza del producto cárnico (Tabla I.2) (Hernández-Jover et al., 1996a; Saccani et al., 2005; Ruiz-Capillas et al., 2007 a, b; Delgado-Pando et al., 2011b; Delgado-Pando et al., 2012)

Los procesos de fermentación y curado están principalmente asociados a la formación de aminas biógenas (Figura I.2). Los productos cárnicos fermentados y curados presentan la mayor cantidad y diversidad de aminas biógenas (Tabla I.2). El origen de las aminas biógenas en estos productos cárnicos puede ser debida en parte a la materia prima, sin embargo la mayor proporción se origina a lo largo de las distintas etapas del procesado (Vidal-Carou et al., 1990; Maijala et al., 1995; Bover-Cid et al., 2001b). Algunos autores han observado mayores concentraciones de aminas biógenas durante las etapas de fermentación que durante el secado-curado que podría explicarse por una disminución de la a_w, que limita el crecimiento microbiano (Maijala & Eerola, 1993; Eerola et al., 1996; Hernández-Jover et al., 1997; Treviño et al., 1997).

Las principales aminas biógenas en este tipo de productos son tiramina, putrescina y cadaverina que pueden alcanzar niveles superiores a 850 mg/kg (Tabla I.2). Factores asociados con este tipo de procesos como la temperatura de fermentación (entre 7 y 28 ºC), microorganismos presentes, aditivos usados durante la elaboración, etc., condicionan la formación de aminas biógenas. **La temperatura de fermentación** favorece el crecimiento de microorganismos y con ello la producción de aminas biógenas; por lo tanto el control de esta temperatura podría ser un parámetro muy útil para prevenir la formación de aminas biógenas en derivados fermentados al establecer condiciones poco favorables para el crecimiento de bacterias productoras de estas aminas (Maijala et al., 1995; Eerola et al., 1998). De hecho, Kranner et al. (1991) han observado una reducción en la formación de la histamina a temperaturas de fermentación entre 7 y 18 ºC.

Como ya se ha comentado, el empleo de **cultivos iniciadores** no productores de aminas biógenas también puede ser una estrategia para limitar la producción de aminas biógenas (Komprda et al., 2001, 2009; Gençcelep et al., 2007; Hu et al., 2007; Coloretti et al., 2008; Gücükoglu et al., 2010; Lu et al., 2010). Por ejemplo, se ha observado que *Pediococus pentosaceus*, *Staphylococcus xylosus* y *Lactobacillus sakei*, pueden inhibir la producción de aminas biógenas en productos fermentados (Bover-Cid et al., 2001a).

Otros factores, como el **diámetro** de productos fermentados puede también influir sobre la formación de aminas biógenas durante la maduración. Se observó una mayor

concentración de aminas biógenas en salchichas con mayor diámetro en comparación con las de menor diámetro. También se ha observado una presencia más elevada de aminas biógenas en la parte central que en los extremos del producto (Bover-Cid et al., 1999a; Suzzi & Gardini, 2003; Ruiz-Capillas & Jiménez-Colmenero, 2004). Este hecho también está relacionado con el nivel de a_w que influye en el crecimiento de microorganismos. Un mayor diámetro de salchicha está asociado a una mayor a_w (Eerola et al., 1996).

Los **ingredientes utilizados** en la preparación de productos fermentados, como los azúcares, también son un factor importante en la formación de aminas biógenas, si bien su magnitud va a depender del tipo de ingrediente, concentración, etc. Sin embargo, a este respecto los resultados han sido contradictorios. Vandekerckhove (1977) encontró que ninguno de los distintos tipos de azúcares empleados en la maduración de productos fermentados influyó en la formación de las aminas biógenas, por contra otros autores han observado que azúcares como la glucosa limitaba la formación de aminas biógenas en estos derivados debido a la acidificación que producen durante el proceso de fermentación (Bover-Cid et al., 2001b). La concentración óptima de glucosa para la producción de enzimas aminoácido decarboxilasa se ha señalado entre 0,5 y 2%, mientras que concentraciones superiores al 3% inhiben la producción de aminas biógenas debido a su efecto en el descenso del pH (Kranner et al., 1991; Masson & Montel, 1995; Hernández-Jover et al., 1997; Ruiz-Capillas & Jiménez-Colmenero, 2004).

Las tecnologías de procesado y conservación de los productos cárnicos, como por ejemplo la aplicación de **altas presiones** también se ha visto que influye sobre la proliferación microbiana y por lo tanto en la producción de aminas biógenas. Distintos estudios han puesto de manifiesto que la aplicación de altas presiones a productos cárnicos (jamón, chorizo, salchichas tipo frankfurt, etc.) suponen una disminución en los recuentos bacterianos y los niveles de aminas biógenas producidas en estas condiciones (Garriga et al., 2005; Latorre-Moratalla et al., 2007; Ruiz-capillas et al., 2007 b, c). Así en estudios de productos fermentados como el chorizo, tratados con altas presiones, se han observado altas concentraciones de agmatina relacionados con elevados contenidos de enterobacterias y bacterias acido lácticas. Sin embargo, estos mismos productos presentaban niveles bajos de tiramina en comparación con las muestras sin tratamiento (Roig-Sagués & Eerola, 1997). Ruiz-Capillas et al. (2007c)

observaron que la presurización de chorizo a 350 MPa durante 15 min a 20 ºC, originaban una disminución en los niveles de tiramina, putrescina, cadaverina y espermina, incrementando los de espermidina.

La **irradiación** de carne y de productos cárnicos se ha asociado también a un descenso en los recuentos microbianos y la producción de aminas biógenas (Min et al., 2007; Wei et al., 2009; Rabie et al., 2010). En carne de vacuno y de cerdo inoculados con microorganismos productores de aminas biógenas *Bacillus cereus, Enterobacter cloacae y Alcaligenes faecalis* y tratados con irradiaciones gama a 2 kGy, se observó una disminución significativa en los niveles de aminas biógenas al cabo de los 24 h a 4 °C (Min et al., 2007). Así mismo se observaron reducciones en los valores de tiramina, espemina, espermidina y putrescina con contenidos estables de cadaverina y β-feniletilamina en salchichas pepperoni con tratamientos entre 5 y 20 kGy (Kim et al., 2005).

Las tecnologías de conservación también constituyen un factor determinante en la producción de aminas biógenas. La **temperatura** influye en el crecimiento microbiano como se ha comentado anteriormente (Karovičová & Kohajdová, 2005). La temperatura óptima de conservación para inhibir el crecimiento bacteriano en carne y productos cárnicos está en torno a 2° C (Kim et al., 2002; Ruiz-Capillas & Jiménez-Colmenero, 2004; Rodtong et al., 2005).

El empleo de **las atmósferas protectoras** se ha visto que también influye en la formación de aminas biógenas en carne y productos cárnicos, estando su efecto asociado al tipo y concentración de gases en el interior del envase. En este sentido, Gallas et al. (2010) han observado que una atmósfera con mayor proporción de oxígeno (75%) reduce altamente la producción de aminas biógenas en carne de pollo en comparación con una atmósfera modificada compuesta de una proporción mayor en nitrógeno (75%). Sin embargo, no se apreciaron diferencias en la proliferación bacteriana y en la producción de aminas biógenas en productos fermentados, tipo chorizo, conservados a vacío en refrigeración y con mezcla de atmósferas con 20/80% CO_2/N_2 y con 30/70% CO_2/Ar (Ruiz-Capillas et al., 2011). Tampoco se observaron diferencias significativas en la producción de aminas biógenas en salchichas frescas conservadas en condiciones similares (Ruiz-Capillas & Jiménez-Colmenero, 2010).

Tabla I.2. Niveles de aminas biógenas en carne y productos cárnicos (adaptada de Ruiz-Capillas & Jiménez-Colmenero, 2004)

	Biogenic amines (mg/kg)								
Products	**Histamine**	**Tyramine**	**Cadaverine**	**Putrescine**	**Tryptamine**	**β-phenylethylamine**	**Spermidine**	**Spermine**	**References**
Fresh and cooked products									
Fresh Pork raw	Nd-6	Nd-56	Nd-13.3	Nd-16	—	Nd-5.6	Nd–37	19–67.1	Halász et al., 1994; Saccani et al., 2005; Favaro et al., 2007; Min et al., 2007
Fresh pork stored at 6-8 ºC for 8 days	Nd-7	Nd-75	Nd-130	Nd-80	—	—	4-6	21-33	Hernández-Jover et al., 1996a
Fresh Beef raw	Nd-2.7	Nd-38	Nd-1.92	Nd-5.5	20.75-23.38	2.6-6.1	1.5–4.2	9.67–44.6	Min et al., 2007; Galgano et al., 2009
Minced beef and pork	Nd-8	Nd-39	Nd-96	Nd-69	—	—	Nd-5	14–39	Wortberg & Woller, 1982
Raw ground beef at 4 ºC for 12 days	31.8	12.4	Nd	74.1	—	—	113.3	331.3	Sayem-El-Daher et al., 1984
Pork stored at 5 ºC for 15 days	9.9	—	43.0	18.9	—	—	3.1	31.2	Halász et al., 1994
Fresh Pork stored at −20 ºC for 15 days	0.5	—	41.2	11.2	—	—	4.3	42.8	Halász et al., 1994
Leg Lamb stored at 5 ºC for 5 days	—	—	1.3	3.3	—	—	—	—	Edwards et al., 1983
Broiler chicken	0-53	1-200	1-230	1-190	0-19	9-22	9.8-14	75-82	Rokka et al., 2004
Chicken breast	Nd-19.2	Nd-17.4	Nd-252.7	Nd-409.6	—	—	4.8-8.7	11.2-53.3	Silva & Gloria, 2002; Balamatsia et al., 2006
Fresh Beef packaged (PVC PLA, Matter-Bi-1/2 films)	1.72-1.94	4.55-5.01	1.66-1.77	1.84-1.96	19.96-24.99	—	2.13-2.21	10.05-11.70	Galgano et al., 2009
Chicken breast stored under MAP (25% CO_2 and 75% N_2)	Nd-1.8	Nd-3.2	Nd-42.4	Nd-72.5			6.3-7.6	15.3-17.9	Gallas et al., 2010
Chicken breast stored under MAP (25% CO_2 and 75% O_2)	Nd	Nd	Nd-9.5	Nd-29.8	—	—	5.9-7.7	14.9-17.8	Gallas et al., 2010
Chicken breast stored under MAP (30% CO_2 and 70% N_2)	Nd-26.8	0.3-8.9	8.5-223.7	48-354	—	—	7.8-13.2	31.5-56.6	Balamatsia et al., 2006
Vacuum packed fresh beef at 1 ºC for 7 weeks	3	6	54	18	—	—	3	25	Edwards et al., 1987
Fresh pork (CO_2) at −1.5 ºC for 13 weeks	16	60	68	20	—	40	9	600	Nadon et al., 2001
Pork stored in CO_2/air at 2 ºC	—	0.7	39.6	6.6	Nd	—	3.2	26.5	Ordoñez et al., 1991
Vacuum packed sterile beef at 1 ºC for 8 weeks	—	—	0.3	1	—	—	—	—	Edwards et al., 1985
Vacuum packed beef at 1 ºC for 7 weeks	—	—	90–158	22–110	—	—	—	—	Edwards et al., 1985
Fresh vacuum packed beef at 1ºC for 120 days	Nd	286	—	—	49	Nd	—	—	Smith et al., 1993
Fresh pork sausages vacuum packed and under MAP	<1	Nd-2.18	Nd	<1		Nd-2.42	2.46-3.36	21.8-26.1	Ruiz-Capillas & Jiménez-Colmenero, 2010
Precooked chicken meat	1.9-25.11	0.3-18.8	0.2-14.8	0.8-202.6	—	—	66.7-273.7	11.5-46.3	Patsias et al., 2006
Precooked chicken meat under MAP	3.1-37.5	0.1-8.8	1.1-21.8	0.5-90.4	—	—	114.2-318.7	19.9-67.4	Patsias et al., 2006
Smoked turkey breast fillets at 4 ºC	Nd-32.9	Nd-25	Nd-2.5	Nd-2.5	Nd-4.1	—	Nd-3.5	Nd-30	Ntzimani et al., 2008
Cooked Spanish meat products "Morcilla"	—	Nd-8.4	—	—	—	—	—	—	Santos et al., 1985
Bologna sausage	Nd–6	Nd–29	Nd–57	Nd–29	—	—	1.5–4	15–36	Wortberg & Woller, 1982
Ground meat and processed meat products	5.9–16.1	1.5–35.5	1.3–10.2	0.3–12.3	2.3–13.5	Nd-2.5	1.6–5.1	2.1–6.1	Durlu-Özkaya et al., 2001

Nd: not detected.

Continuación Tabla I.2. Niveles de aminas biógenas en carne y productos cárnicos (adaptada de Ruiz-Capillas & Jiménez-Colmenero, 2004)

	Biogenic amines (mg/kg)								
Products	**Histamine**	**Tyramine**	**Cadaverine**	**Putrescine**	**Tryptamine**	**β-phenylethylamine**	**Spermidine**	**Spermine**	**References**
Mortadella	Nd-4.8	Nd-66.0	0.6–7.0	Nd-3.9	Nd-1.0	Nd-1.4	1.9–8.9	7.8–32.2	Hernández-Jover et al., 1997
Cooked ham	Nd-11	Nd-108	Nd-12	Nd-139	nd	Nd-2	1–18	18.1–25.4	Hernández-Jover et al., 1997; Saccani et al., 2005
Cooked meat products "Chopped"	1.40	17.60	—	—	—	—	—	—	Vidal-Carou et al., 1990
Frankfurters sausages	Nd–1.5	2.57–6.1	1.18–3.9	Nd–0.1	Nd	1.78–7.97	0.48–1.3	19.32-–24.2	Ruiz-Capillas et al., 2007b; Delgado-Pando et al., 2011a
Cooked ground beef at 4 ºC for 12 days	85.4	25.1	Nd	85.4	—	—	189.0	382.1	Sayem-El-Daher et al., 1984
Cooked sliced ham	0.75	1.46	0.03	0.14	—	—	2.36	27.40	Ruiz-Capillas et al., 2007a
Pork liver pâté	1.21–2.28	0.70–2.12	8.98–23.17	1.95–6.23	Nd	Nd	6.38–13.23	38.49–59.62	Delgado-Pando et al., 2012
Cooked meat products "Butifarra catalana"	0.50–0.70	14.95–151.8	—	—	—	—	—	—	Vidal-Carou et al., 1990
Dry, cured, ripened and fermented products									
Portuguese ripened sausage (choriço)	4.2–7.6	26.7–36.2	668.4–1807	1506–1672	838.9–860.5	87.7–125.9	30.9–43.6	113.3–125.9	Roseiro et al., 2010
Ripened Chorizo	Nd-361.7	76.5–477.8	3.9–812.5	31.6–361.9	5.7–65.1	Nd-7.7	0.5–7.9	15.4–37.8	Bover-cid et al., 1999b, 2000a, 2001 a, b, 2006; Gonzalez Fernandez et al., 2003; Ruiz-Capillas & Jiménez-Colmenero, 2004; Ruiz-Capillas et al., 2007c; Latorre-Moratalla et al., 2010b; Casquete et al., 2012
Turkish soudjouck	—	Nd-349	—	Nd-412	—	Nd	—	—	Ayhan et al., 1999
Ripened Salchichón	2.35–87.3	67.5–465.2	2.1–68.5	85.9–184.5	12.9–47.4	2.7–34.7	2.5–3.9	8.9–297.7	Hernández-Jover et al., 1997c
Ripened Jamón serrano	1.95-128.40	0.45–69.50	—	—	—	—	—	—	Vidal-Carou et al., 1990
Ripened Lomo embuchado	7.10–78.10	60.50–99.25	—	—	—	—	—	—	Vidal-Carou et al., 1990
Ripened Sobrasada	3.10–14.25	14.15–77.55	—	—	—	—	—	—	Vidal-Carou et al., 1990
Italian ripened sausage (Soppressata)	0–101	0–557	0–271	0–416	—	0–20	2	17	Parente et al., 2001; Favaro et al., 2007
Sucuk	Nd-136	2.4-676	Nd-199	Nd-364	Nd-82.3	Nd-153.6	Nd-12	Nd-16.4	Gençcelep et al., 2007; Kurt & Zorba, 2010
Egyptian ripened dry sausage	7.5–800	9.5–850	5.6–2600	12–1600	2.5–33.2	1.5–80.7	5.3–11.7	1.5–5.2	Shalaby, 1993, 1996; Rabie et al., 2010
Portuguese dry fermented sausage	24.5	252.9	720.9	466.1	7.9	53.8	2.9	32.2	Roseiro et al., 2006
Dry fermented sausages	0–314.3	3–626.8	0–790	0–580	5–91	<1–75	<1–26	8–59	Hernández-Jover et al., 1996b, 1997; Eerola et al., 1998; Bover-cid et al., 2000b; Ruiz-Capillas & Jiménez-Colmenero, 2004; Saccani et al., 2005; Miguélez arrizado et al., 2006; Latorre-Moratalla et al., 2008; Komprda et al., 2009; Lu et al., 2010; Ruiz-Capillas et al., 2011; Tabanelli et al., 2012
Dry cured fermented pork loins	Nd	—	Nd–39.6	0.6-28.6	Nd–49.2	—	Nd	4–5.8	Stadnik & Dolatowski, 2012
Dry-cured ham	Nd-7	4-274	Nd-64	1-237	—	1-19	1-37	16-138	Saccani et al., 2005; Favaro et al., 2007; Virgili et al., 2007; Martuscelli et al., 2009
Dry-cured belly	114	295	6	331	—	—	1.3	16	Favaro et al., 2007
Dry cured lacón	4.01–5.94	1.62–6.11	5.20–39.15	Nd–6.67	33.80–62.83	4.33–22.08	7–9.75	18.71–30.03	Lorenzo et al., 2007
Spanish ripened sausage "Mini-salami"	4–16	3–12	Nd	42–139	—	—	5–10-	25	Treviño et al., 1997
Spanish ripened sausage "Fuet"	15.2	156.9	367	64.7	10.0	10.1	10.3	30.6	Bover-Cid et al., 1999a

Nd: not detected.

I.1.3. Importancia de las aminas biógenas

La determinación de aminas biógenas en los alimentos en general, y en los productos cárnicos en particular, tiene un doble interés, por un lado por su efecto toxicológico y por otro por la posibilidad de ser empleados como índices de calidad.

I.1.3.1. Interés toxicológico

La presencia de aminas biógenas en los alimentos presenta efectos fisiológicos aunque en algunos casos constituye un potencial problema de salud pública debido a sus posibles efectos toxicológicos (Tabla I.3). El consumo de alimentos con altas concentraciones de aminas biógenas ha sido relacionado en determinadas ocasiones con problemas toxicológicos en los consumidores. La tiramina y la histamina son las aminas biógenas que presentan un mayor potencial tóxico (Tabla I.3).

El consumo de alimentos con elevada concentración de **tiramina** se manifiesta con fenómenos de hipertensión, taquicardia, aumento de la respiración y glucemia, liberación de norepinefrina, hemorragia cerebral y cardíaca, etc., conocidos como la "reacción de queso". Esto es así porque originalmente estos síntomas se asociaban al consumo de queso con elevadas concentraciones de tiramina (Tabla I.3) (Bodmer et al., 1999; Alberto et al., 2002; Ruiz-Capillas & Jiménez-Colmenero, 2004; Karovičová & Kohajdová, 2005; Önal, 2007; Standarová et al., 2008). Sin embargo, esta amina también está presente en carne y productos cárnicos por lo que se debe prestar especial atención a su efecto en estos alimentos.

La intoxicación alimentaria más frecuente por aminas biógenas es la causada por la **histamina**. Es conocida como la intoxicación histamínica e "intoxicación por escómbridos", por su relación con el consumo de pescados de esta familia, como atún, caballa y sardinas (Santos, 1996) que contienen elevadas concentraciones del aminoácido histidina. La intoxicación se produce por acumulación de histamina en el tracto intestinal a partir de los alimentos y causa un incremento histamínico en el plasma que lleva a la histaminosis. Los síntomas de intoxicación histamínica son de cuatro tipos: (1) A nivel del sistema circulatorio (cefalea, hipotensión, arritmia y anafilaxis), (2) de la piel (picor, urticaria y enrojecimiento), (3) del sistema respiratorio (obstrucción nasal y brococonstricción) y (4) del tracto gastrointestinal (dolor abdominal, diarrea, nauseas y vómitos). Recientemente se ha relacionado también la migraña con altos niveles de las aminas biógenas, y particularmente de la histamina (Vidal-Carou et al., 2010) (Figura I.3 y Tabla I.3).

Tabla I.3. Efectos fisiológicos y potenciales toxicológicos de aminas biógenas (adaptada de Ladero et al., 2010)

Aminas biógenas	Efectos fisiológicos	Efectos toxicológicos
Tiramina	Neurotransmisor; favorece la vasoconstricción periférica; aumenta la presión arterial, la respiración y la glucemia; libera la norepinefrina; incrementa el nivel de azúcar en la sangre (glucemia); interviene en la formación de nitrosaminas, etc.	Dolor de cabeza, migraña, desorden neurológico, nausea, vómitos, desorden respiratorio, hipertensión y crisis hipertensiva (reacción de queso), parálisis de las extremidades, taquicardia, provoca hemorragia cerebral y cardiaca, etc.
Histamina	Neurotransmisor; psicoactiva; hormona local; secreciones gástricas ácidas; interviene en el crecimiento y la diferenciación celular, el ritmo circadiano, la regulación de la temperatura corporal, la ingesta de alimentos, el aprendizaje y la memoria, la respuesta inmune, las reacciones alérgicas y la síntesis de noradrenalina y adrenalina; participa en los procesos inflamatorios, en la cicatrización de tejidos, en la regulación de la circulación local; tiene un efecto vasodilatador de los vasos sanguíneos, capilares y arteriales; disminuye la presión arterial, etc.	Dolor de cabeza, migraña, hipotensión, arritmia, taquicardia, extrasístoles, palpitaciones, anafilaxia, sofocos, edemas, sudoración, obstrucción nasal, brococonstricción, bronco espasmo, enrojecimiento facial, erupciones cutáneas, urticaria, picor, dolor gastrointestinal, diarrea, mareos, vómitos, nauseas, dificultad respiratoria, trastornos de la presión arterial y de la neurotransmisión, etc.
Putrescina Cadaverina	Neurotransmisor; psicoactiva; regula la expresión genética; interviene en la maduración y absorción intestinal, el crecimiento y la diferenciación celular y la formación de nitrosaminas; disminuye la presión arterial y el catabolismo de la histamina y la tiramina, etc.	Taquicardia, hipertensión, efectos carcinogénicos, rigidez mandibular, bradicardia, potencia el efecto de otras aminas biógenas, provoca trastornos de la neurotransmisión, etc.
Serotonina	Influye en el volumen y frecuencia cardiaca; tiene un efecto vasodilatador de los vasos sanguíneos, capilares y arteriales; estimula la musculatura lisa (estomago y intestino); interviene en el metabolismo de los hidratos de carbono; es un neurotransmisor, etc.	Dolor de cabeza, hipotensión, sofocos, dolor gastrointestinal, edemas, migrañas, etc.
β-feniletilamina	Favorece la vasoconstricción periférica	Hipertensión, migrañas
Triptamina	Favorece la vasoconstricción periférica	Hipertensión

La putrescina y cadaverina también presentan elevada toxicidad cuando se administran en altas dosis (Til et al., 1997). Además, estas dos diaminas favorecen la absorción intestinal y disminuyen la desintoxicación de histamina y tiramina potenciando así su toxicidad (Landete et al., 2007; Önal, 2007) (Tabla I.3). Por otro lado, putrescina y cadaverina participan en la formación de nitrosaminas, compuestos potencialmente cancerígenos (Al Bulushi et al., 2009). Este fenómeno es especialmente

importante en productos cárnicos a los que se adicionan nitratos y nitritos y en los tratados térmicamente ya que favorece la interacción entre aminas biógenas y nitritos para formar nitrosaminas (Patterson & Mottram, 1974). Dado que son muy pocos los estudios sobre la posible relación entre el contenido de aminas biógenas y la formación de nitrosaminas, se hace difícil valorar el verdadero riesgo de formación de las mismas a partir de las aminas biógenas en los productos cárnicos.

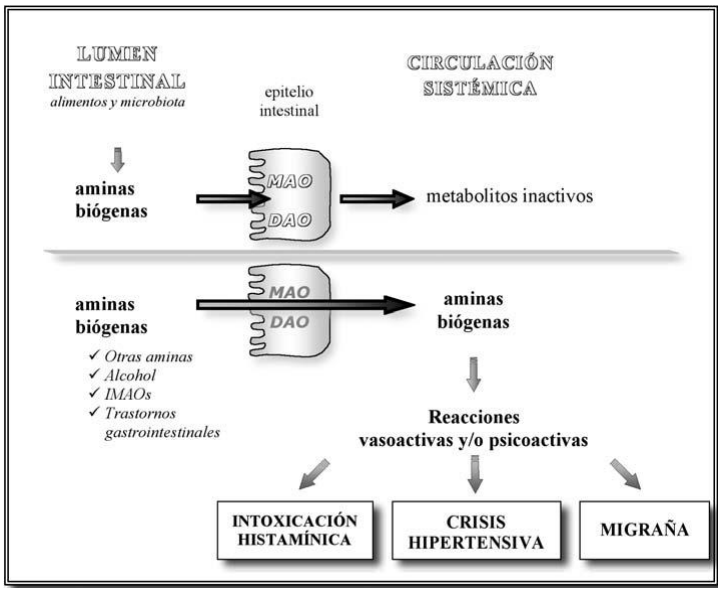

Figura I.3. Efectos tóxicos de las aminas biógenas y factores potenciadores (Bover-cid et al., 2005)

Sin embargo, se debe tener en cuenta que en circunstancias normales, el organismo humano dispone de sistemas de desintoxicación de estas aminas biógenas principalmente a nivel intestinal debido a la acción de la monoamino oxidasa (MAO; CE 1.4.3.4), diamina oxidasa (DAO; CE 1.4.3.6) y poliamina oxidasa (PAO; CE 1.5.3.11) (Figura I.3) (Ruiz-Capillas & Jiménez-Colmenero, 2004; Bover-Cid et al., 2005). Por lo tanto, la toxicidad de estas aminas biógenas depende de la eficiencia del sistema de desintoxicación que puede verse alterado por diferentes factores o circunstancias, entre ellos el consumo de IMAO/IDAO (inhibidores de monoamino oxidasa y de la diamina oxidasa), alcohol, deficiencia del sistema inmune, enfermedades gastrointestinales, etc. (Bardócz, 1995). La alteración de este sistema expone al individuo a un alto riesgo de toxicidad en presencia de las aminas biógenas (Figura I.3). De hecho, escasa cantidad de tiramina podría provocar fuertes migrañas, con hemorragia intracraneal en pacientes tratados con IMAO clásicos (McCabe-Sellers et al., 2006), mientras que una ingesta de tiramina entre 50 y 150 mg es más tolerada por

pacientes tratados con una nueva generación de IMAO, los denominados RIMA (inhibidor reversible de MAO-A) (EFSA, 2011).

I.1.3.2. Legislación

A pesar de la importancia de las aminas biógenas, no hay legislación específica para el establecimiento de límites legales de su presencia en productos cárnicos. Sólo existe una legislación específica para la histamina, y no para las demás aminas biógenas, y esta es principalmente para pescado. Además, se aprecia cierta discrepancia en los niveles establecidos. La legislación europea (Directiva 91/439/CEE) permite un límite en los valores de histamina en productos de la pesca de 100 mg/kg. Mientras que la agencia de Food and Drug Administration (FDA, 2011) ha establecido el límite de tolerancia de histamina en alimentos en general, en 50 mg/kg.

Por otro lado, la Autoridad Europea para la Seguridad Alimentaria (EFSA, 2011) ha fijado un límite para productos cárnicos, a niveles de 50 mg/kg para la histamina y de hasta 600 mg/kg para la tiramina.

Otras instituciones oficiales, como el Instituto Holandés de investigación de productos lácteos también han establecido un límite de entre 400-800 mg/kg para la tiramina (ten Brink et al., 1990). En cambio otros autores limitaron la tiramina a un rango de 100-800 mg/kg en productos cárnicos (Stadnik & Dolatowski, 2010). Por otro lado, un valor de 30 mg/kg para β-feniletilamina ha sido señalado como dosis tóxica en los productos alimentarios incluyendo los productos cárnicos (Gardini et al., 2001).

En cualquier caso cabe concluir que fijar los límites es una tarea difícil porque el efecto tóxico de las aminas biógenas no depende solamente de su presencia, sino también de su interacción con otros compuestos y de la eficacia específica de los mecanismos de desintoxicación del individuo (Halász et al., 1994; Eerola et al., 1997; Gardini et al., 2001; Ruiz-Capillas & Jiménez Colmenero, 2004). Se debe tener en cuenta que, como se ha comentado anteriormente, diferentes factores pueden influir en la toxicidad de las aminas biógenas lo que condiciona el limite toxicológico de éstas (Vidal-Carou et al., 1990; Ruiz-Capillas & Jiménez-Colmenero, 2004). Así Vidal-Carou et al. (1990) determinaron que el 63% de muestras de salchichón y el 64% de chorizo contenían tiramina en cantidades suficientes como para originar un efecto toxicológico en el consumidor que estaba tomando IMAOs. Estos autores también han estimado que una concentración de 6 mg/kg de tiramina puede ser considerada como tóxica si es ingerida simultáneamente con IMAOs. Por otra parte, Santos et al. (1985) han sugerido

que el consumo de 100 g de diversos productos cárnicos (chorizo, salami, lomo curado, morcilla o hamburguesa, etc.) que contienen más de 8,4 mg/kg de tiramina puede ser tóxico cuando se consumen junto con IMAOs. En ausencia de IMAOs, ninguno de esos productos llegan a causar problemas cuando son consumidos de manera moderada. No obstante, esto siempre va depender de la susceptibilidad del individuo.

I.1.3.3. Aminas biógenas como índices de calidad

La determinación de aminas biógenas en los alimentos no es solamente importante desde un punto de vista toxicológico sino también por su empleo como indicadores de calidad.

Las aminas biógenas han sido empleadas para establecer índices de calidad en diferentes alimentos con el objetivo de informar del nivel de frescura y/o deterioro de los productos.

En la Tabla I.4 se señalan algunos de los índices basados en la presencia de aminas biógenas empleados en carne y productos cárnicos. El más tradicional para la evaluación de la calidad de los alimentos fue propuesto por Mietz & Karmas (1977a), como indicador de la descomposición del pescado. Este índice se basa en el incremento de los niveles de putrescina, cadaverina e histamina, y la disminución de los niveles de espermidina y espermina, a lo largo de la conservación del pescado. Con unos rangos entre 0 y 1 mg/kg, indicativos de buena calidad del pescado, de entre 1 y 10 mg/kg como límite de tolerancia, y de más de 10 mg/kg indicando descomposición del producto. Sin embargo, en el caso de carne y productos cárnicos este índice no ha proporcionado buenos resultados, por lo que se han propuesto distintas alternativas (Tabla I.4). Así, Wortberg & Woller (1982) han sugerido un índice de aminas biógenas (IBA) que consiste en la suma de putrescina, cadaverina, histamina y tiramina, eliminando la espermidina y la espermina del índice de Mietz & Karmas (1977a).

Hernández-Jover et al. (1996a) han sugerido límites de IBA <5 mg/kg para la carne fresca de buena calidad, entre 5 y 20 mg/kg para una carne fresca aceptable pero con señales de inicio del deterioro, entre 20 y 50 mg/kg para una baja calidad de carne y finalmente > 50 mg/kg para una carne deteriorada. Otros autores sugieren una combinación de putrescina y cadaverina como índice de aceptabilidad en carne fresca porque sus concentraciones aumentan durante el deterioro y se correlacionan bien con el crecimiento microbiano (Edwards et al., 1985). Vinci & Antonelli (2002) también han propuesto concentraciones de cadaverina y tiramina para evaluar el deterioro de carne

de vacuno y de pollo durante el almacenamiento. De forma individual, la tiramina ha sido también ampliamente usada como indicador de calidad para carne de vacuno envasada a vacío y para jamón cocido sujetos a procesos de alta presión (Edwards et al., 1987; Ruiz-Capillas et al., 2007a).

Poliaminas como espermidina y espermina también han sido propuestas como índices para la evaluación de la calidad de la carne de pollo durante el almacenamiento en refrigeración (Silva & Gloria, 2002).

La aplicación de estos índices de calidad en los productos fermentados sin embargo, presentan importantes dificultades debido al número de factores involucrados en la formación de aminas biógenas, entre ellos la presencia de cultivos iniciadores, empleo de aditivos, etc. (Leuschner et al., 1998).

Tabla I.4. Ejemplos de índices de calidad basados en la presencia de aminas biógenas en carne y productos cárnicos (adaptada de Ruiz-Capillas y Jiménez-Colmenero, 2004)

Products	**Biogenic amines**	**References**
Bologna sausage and minced beef and pork	BAI= putrescine+cadaverine+histamine+tyramine	Wortberg & Woller, 1982
Pork meat at 6-8ºC	BAI= putrescine+cadaverine+histamine+tyramine	Hernández-Jover et al., 1996b
Chicken breast and thigh	Spermidine/Spermine	Silva & Gloria, 2002
Chicken Breast stored in MAP	Putreescine+cadaverine+tyramine	Balamatsia et al., 2006
Smoked Turkey breast fillets	Tryptamine, histamine and tyramine	Ntzimani et al., 2008
Raw and cooked ground beef	Putrescine, tyramine	Sayem-El-Daher et al., 1984
Ground meat and processed meat products	BAI= putrescine+cadaverine+histamine+tyramine	Durlu-Özkaya et al., 2001
Meat and meat products	BAI= putrescine+cadaverine+histamine+tyramine	Nowak & Czyzowska, 2011
Fresh meat	Tyramine and cadaverine	Vinci & Antonelli, 2002
	Tyramine, putrescine and cadaverine	Krizek et al., 1995; Rokka et al., 2004
	Putrescine and cadaverine	Slemr, 1981
Vacuum packed beef at 1ºC	Putrescine and cadaverine	Edwards et al., 1985
Vacuum packed beef at 1ºC	Tyramine	Edwards et al., 1987; Smith et al., 1993
fresh beef meat packed in aerobic atmosphere with biopolymers	Tyramine and cadaverine	Galgano et al., 2009
Wrapped and unwrapped fresh meat (pork, beef and rabbit)	Putrescine and cadaverine	Guerrero-Legarreta & Chavez-Gallardo, 1991
Cooked Spanish products "Jamón york", "chopped" and "butifarra"	Putrescine and cadaverine	Vidal-Carou et al., 1990
Cooked ham	Tyramine	Ruiz-Capillas et al., 2007a
Dry sausages	Tyramine, histamine, putrescine and cadaverine	Eerola et al., 1996-1998
Fermented sausages	Tyramine, histamine, putrescine and cadaverine	Ruiz-Capillas & Jimenez-Colmenero, 2004

I.1.4. Determinación de las aminas biógenas

Numerosos procedimientos analíticos se han desarrollado para la determinación de las aminas biógenas en diferentes alimentos (Hurst, 1990; Hernández-Jover et al., 1996b; Önal, 2007; Ruiz-Capillas & Jiménez-Colmenero, 2009). Estos abarcan desde métodos colorimétricos y fluorimétricos simples hasta los métodos más sofisticados, como los métodos cromatográficos (CG, HPLC, HPTLC, etc.).

Los primeros **métodos colorimétricos** publicados por la AOAC (1995a) (Nº 957.07) se basaron en la extracción de la histamina con metanol y su reacción con ninhidrina para su cuantificación. Estos métodos, no se usan hoy en día debido a que son tediosos, requieren especial atención en los detalles del procedimiento y purificación previa de la muestra (Bateman et al., 1994; Patange et al., 2005).

Los **métodos fluorimétricos,** descritos por la AOAC (1995b) (977.13), han sido usados igualmente para determinar histamina en los alimentos. El método se basa en la extracción con metanol de la histamina, posteriormente derivatizada con o-phtalaldehido (OPA) para producir un compuesto fluorescente que es determinado mediante un fluorimetro (Taylor et al., 1978). Este método presenta demasiadas interferencias y sólo permite determinar histamina.

Los **métodos enzimáticos** empleados se fundamentan en la utilización de enzima diamina-oxidasa (DAO) que cataliza la conversión de la histamina a imidazol acetaldehído con producción simultánea de peróxido de hidrógeno. La adición de una segunda enzima, la peroxidasa, y de un leuco-cristal incoloro, provoca una oxidación del leuco-cristal que origina un compuesto de color morado que se mide mediante espectrofotometría. La intensidad del color es proporcional a la cantidad de histamina excedente. Este método ha sido mejorado realizando la lectura en un lector de micro placas (Ben Gigirey et al., 1998). Con el fin de simplificar esta metodología, se han desarrollado también **biosensores** que proporcionan simplicidad y rapidez al sistema. Sin embargo, estos biosensores no son accesibles para análisis rutinarios (Lange & Wittmann, 2002).

A partir de los métodos enzimáticos, se han desarrollado **métodos inmuno-enzimáticos** como ELISA, "immunoassay", "color test", "F.L.O.R.I.D.A.", etc. para la medición de la histamina en los alimentos. Estos métodos se encuentran en el mercado, y se basan en una reacción colorimétrica que emplea anticuerpos. Estos anticuerpos específicos de las enzimas DAO se emplean para fijar estas enzimas, previamente marcadas con una molécula coloreada, en los pocillos del equipo inmuno-enzimático

usado. Se mide por espectrofotometría y la coloración obtenida es inversamente proporcional a la cantidad de histamina de la muestra. Sin embargo, no existen en el mercado métodos inmuno-enzimáticos para las demás aminas biógenas (EFSA, 2011).

El **análisis de inyección de flujo (FIA)** también se ha empleado para la determinación de la histamina en alimentos. La muestra se inyecta en el sistema FIA donde se produce la reacción con el OPA, el compuesto resultante se determina mediante un fluorimetro en linea. Los sistemas de análisis de inyección de flujo empleados para la determinación de histamina fueron desarrollados para pescado. Esta técnica permite también determinar solamente la histamina (Hungerford et al, 1990).

La **electroforesis capilar** se ha desarrollado ampliamente en la última década. Las aminas biógenas se extraen con una solución ácida, purificandose antes de la inyección a presión en un tubo capilar, seguido de una separación a temperatura controlada. La detección se realiza mediante un detector de ultravioleta (UV), un diodo de array o un fluorímetro (Figura I.4).

Los métodos más utilizados hoy en día son los **métodos cromatográficos**, principalmente la cromatografía de gases (CG), cromatografía líquida de alta resolución (HPLC), cromatografía de capa fina de alta resolución (HPTLC) y en menor medida la cromatografía en capa fina tradicional (TLC). Estos métodos se han combinado con diferentes sistemas de detección como ultravioleta (UV), fluorescencia, etc., y ofrecen una gran ventaja sobre los métodos señalados previamente permitiendo principalmente el análisis simultáneo de la histamina y otras aminas biógenas en diferentes alimentos.

Los métodos de **TLC** se basan en una extracción de cada amina biógena realizada con metanol o ácido tricloroacético seguida de una migración y separación realizada sobre una fase estacionaria (gel de sílice) en una cámara de cromatografía, con distintos solventes (metanol: amoníaco [20:1] y cloroformo: metanol: amoníaco [2:2:1]). La reacción de derivatizacion se realiza con ninhidrina o fluorescamina o diacetilbenzeno y la detección se realiza comparando la intensidad del color de la amina biógena con sus estándares por detección simultánea en la placa cromatográfica (Liebert & Taylor, 1978 a, b). Se han realizado avances en esta técnica aplicando **HPTLC**. Para ello, las aminas biógenas se extraen con una solución de ácido tricloroacético, siendo purificadas y derivatizadas con cloruro de dansilo. Las aminas biógenas derivatizadas se separan en gel de sílice y se cuantifican por UV (Shalaby, 1994, 1995, 1999; Shakila et al., 2001; Lapa Guimaraes & Pickova, 2004).

La determinación de aminas biógenas por **CG** es un procedimiento empleado desde hace tiempo, realizando una derivatización previa de la muestra (Staruszkiewicz & Bond, 1981). Recientemente, se ha descrito una metodología que se realiza directamente sin derivatisación de las aminas biógenas. Este procedimiento reduce el tiempo de análisis y evita errores que pueden proceder de la derivatización (Hwang et al., 2003).

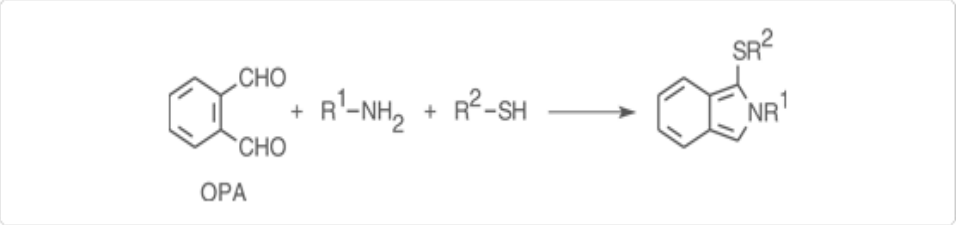

Figura I.4. Reacción de derivatización del O-phthalaldehido (OPA) (LTC, 2013)

Pero probablemente el método más empleado para la determinación de las aminas biógenas sea **HPLC**. Durante años se han propuesto diferentes métodos de HPLC basados en una extracción con ácido tricloroacetico (TCA) o ácido perclórico (PCA) seguida de una derivatización de las aminas biógenas, bien antes o después de la separación en la columna. Los principales derivatizantes utilizados son el cloruro de dansilo y el OPA, para una detección por ultravioleta o fluorescencia en función del derivatizante (Mietz & Karmas, 1977b; Walters, 1984; Rosier & Van Peteghem, 1988; Ritchie & Mackie, 1989; Gouygou et al., 1992; Giorgio et al., 1993; Hernández-Jover et al., 1996b; Malle et al. 1996; Valls et al., 1999; Salazar et al., 2000). Para su separación se han empleado diferentes tipos de columnas C18:0 (Hernández-Jover et al., 1996b) o de intercambio iónico, principalmente catiónico (Ruiz-Capillas & Moral, 2001) (Figura I.5). El empleo de la columna de intercambio iónico tiene ventajas asociadas a su alta selectividad, sensibilidad y eficiencia. Por otro lado, el uso del OPA como derivatizante post-columna aumenta la sensibilidad del método, eliminando posibles interferencias introducidas mediante la derivatización pre-columna, y economizando el tiempo de preparación de las muestras.

Dado que la determinación de aminas biógenas en alimentos, en general, y en productos cárnicos, en particular, es muy importante, resulta necesario disponer de un método que permita cuantificar simultáneamente un elevado número de aminas biógenas, que sea selectivo, de alta sensibilidad y muy eficiente.

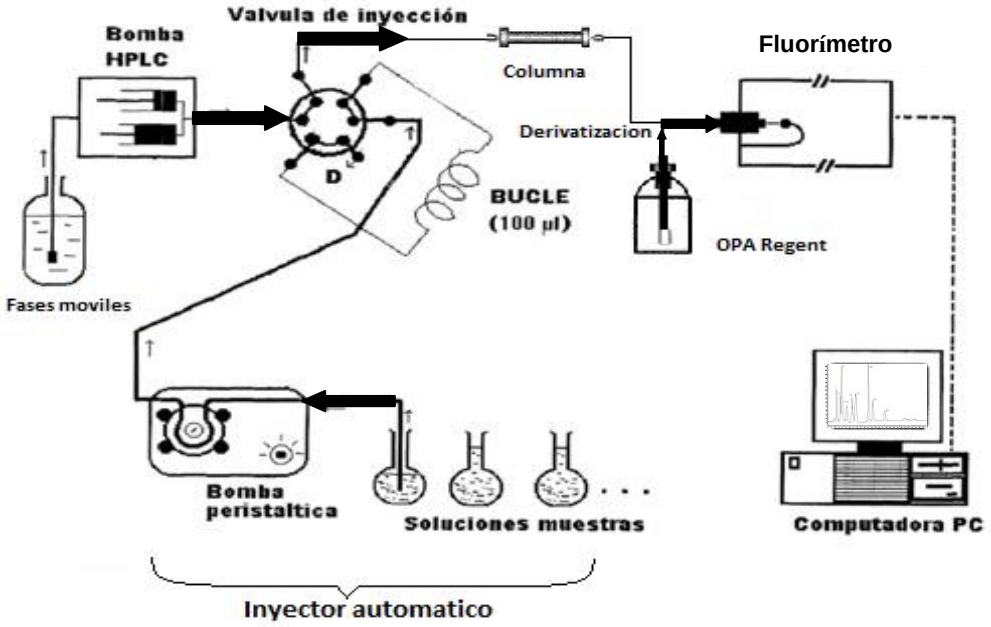

Figura I.5. Esquema de funcionamiento de HPLC con derivatización post-columna de fase de intercambio iónico (Referencia adaptada de Internet)

I.2. PRODUCTOS CÁRNICOS MÁS SALUDABLES

I.2.1. Principales componentes de la carne y productos cárnicos

La carne y los productos cárnicos son componentes esenciales de la dieta tanto en los países desarrollados como emergentes, que concentran y proporcionan un gran número de nutrientes (Tabla I.5). A nivel cuantitativo sus principales componentes, exceptuando el agua, son proteína y grasa, presentando además, importantes aportes de otros nutrientes como vitaminas y minerales, con la ventaja de su elevada biodisponibilidad.

Tabla I.5. Principales componentes de la carne y sus derivados.

Nutrientes	**Vacuno (ternera)[a]**	**Cerdo[a]**	**Chorizo[b]**	**Merguez[c]**
Proteína (g/100g)	17,0-20,7	15,4-21,5	23,8	19,8
Lípidos (g/100g)	5,4-21,0	2,65-29,5	41,7-42,1	23,4
AGS	2,19-8,51	0,90-9,42	15,7	12-12,6
AGMI	2,40-9,33	1,10-12,33	20,1	9,37-9,83
AGPI	0,35-3,28	0,65-4,47	3,79	0,95-1
Colesterol (mg/100g)	59-65	58-72	108	71,4
Minerales (mg/100g)				
Na	61	70-76	1730	891
Fe	1,6-2,1	0,8-1,8	2,8	1,8
Zn	3,3-3,8	1,6-2,5	2,3	2,8
P	170-200	16-170	253	128
Se (µg)	3,0	14,0-32,4	4,9	5
K	350	270-370	479	762
Mg	16-18	16-22	27,5	21,1
Ca	7-8	8-13	35,8	22,9
Vitaminas (mg/100g)				
Tiamina	0,05-0,06	0,57-0,89	0,47	0,05
Riboflavina	0,16-0,22	0,14-0,20	0,26	0,2
Equivalentes de niacina	7,2-8,1	4,1-8,8	4,6	4,7
Vit. B_6	0,25-0,32	0,29-0,45	0,32	0,24
Acido fólico (µg/100g)	8-10	3-5	6,2	20
Vit. B_{12} (µg/100g)	1-2	2-3	0,93	1,45

AGS: Ácidos grasos saturados; AGMI: Ácidos grasos monoinsaturados; AGPI: Ácidos grasos poliinsaturados.
Fuentes: [a] López-lópez, 2010; [b] Moreiras et al., 2005; [c] ANSES, 2008.

I.2.1.1. Proteínas, péptidos y aminoácidos

La carne es una fuente fundamental de proteínas de alto valor biológico, proporciona una balanceada fuente de aminoácidos que atiende a los requerimientos fisiológicos del ser humano. Aporta gran cantidad de aminoácidos esenciales, necesarios para el crecimiento y la reconstitución de los tejidos. También contribuyen a la síntesis de anticuerpos y en consecuencia a la prevención de algunas enfermedades (Romans et al., 1994). Estudios recientes relacionan el consumo de proteína cárnica con una mayor facilidad para perder y mantener el peso corporal gracias a su efecto saciante, resultando útil para reducir el riesgo cardiovascular (Layman et al., 2008; Paddon-Jones et al., 2008). La carne no sólo posee un perfil de aminoácidos próximo a lo ideal, además, aporta una cantidad significativa de los requerimientos diarios de proteínas concentradas en porciones pequeñas de alimento. Dependiendo del porcentaje de humedad y grasa, la carne y sus derivados contienen cantidades de proteínas que puede variar entre 18-24% (Tabla I.5). De la proteína ingerida en la dieta, en torno al 28% (USA) y 30% (Gran Bretaña) es de origen cárnico (Schweitzer, 1995; Higgs, 2000), en España dicha proporción alcanza el 28% (Tabla I.6).

La carne además contiene otros derivados proteicos (péptidos y aminoácidos) con efectos fisiológicos beneficiosos para la salud (Tabla I.7).

I.2.1.2. Lípidos

La grasa de la carne se compone mayoritariamente de lípidos neutros, principalmente triglicéridos (glicerol esterificado con ácidos grasos), fosfolípidos y esteroles (entre ellos colesterol). Aportan gran cantidad de calorías, proporcionan ácidos grasos esenciales, que son precursores de algunos compuestos que regulan funciones fisiológicas (p.e. prostaglandinas), transportan vitaminas liposolubles y dan aislamiento y protección al cuerpo humano (Higgs, 2000; Jiménez-Colmenero, 2007).

Desde el punto de vista de la relación dieta-salud, el tipo de ácidos grasos presentes en el alimento es un factor determinante. Al igual que el contenido en grasa, la composición en ácidos grasos varia con la especie, raza, edad y alimentación (fundamentalmente en porcino y aves), e incluso con su localización anatómica. En general los lípidos de la carne contienen niveles de ácidos grasos saturados (AGS) por debajo del 50% (Tabla I.5). Aunque los ácidos grasos saturados son considerados como los de mayor factor de riesgo por su efecto hipercolesterolémico, no todos ellos actúan de igual manera. Mientras que el esteárico (C18:0) es neutro, el mayor efecto

aterogénico proviene de los ácidos palmítico (C16:0), mirístico (C14:0), presente en cantidades muy pequeñas y laurico, que en conjunto alcanzan niveles de entre el 25-35% del total de ácidos grasos (Higgs & Mulvihill, 2002; Jiménez-Colmenero, 2007). La contribución a la dieta de la grasa animal alcanza los 46% de la totalidad de la grasa ingerida por persona y por día en los países europeos del sur y hasta el 35% en los países del norte de África (FAO, 2007). Mientras que la contribución de los ácidos grasos saturados procedente de la carne y sus derivados alcanza niveles de entre el 13 y el 30% en los países europeos (Tabla I.8). En España la contribución de la carne en grasa total y colesterol alcanzan los 28% y 40 %, respectivamente (Tabla I.6).

Tabla I.6. Perfil nutricional de la dieta media en España y su relación con el consumo de la carne y sus derivados. (Jiménez-Colmenero et al., 2012)

Nutrientes	**Ingesta total por día**	**Nutrientes de la carne**	**Contribución (%) de la carne en la ingesta de cada nutriente**
Calorías (kcal)	2.761	442	16
Proteína (g)	93,5	26,2	28
Carbohidratos (g)	294	0,35	0,1
Grasas (g)	126	35,3	28
Colesterol (mg)	440	175	39,8
Hierro (mg)	13,6	2,3	17
Cinc (mg)	11,1	2,9	26
Magnesio (mg)	313	28,2	9
Sodio (mg)	2.995	808	27
Potasio (mg)	3.507	456	13
Fósforo (mg)	1.534	245	16
Selenio (μg)	108	10,8	10
Tiamina (mg)	1,4	0,51	37
Riboflavina (mg)	1,8	0,32	18
Eq de niacina (mg)	33,7	10,8	32
Vitamina B_6 (mg)	1,7	0,44	26
Vitamina B_{12} (μg)	10,3	1,96	19
Vitamina A: Eq retinol	1.427	499	35
Retinol (μg)	672	356	53
Ácido fólico(μg)	207	12,4	6
Vitamina E (mg)	14,2	0,14	1

Tabla I.7. Ejemplos de derivados proteicos, que poseen efectos fisiológicos beneficiosos para la salud (adaptada de Serrano, 2006)

Derivados proteicos	Origen y/o efecto fisiológico
Arginina	Aminoácido con efecto antioxidante por ser el precursor del óxido nítrico.
Aminoácidos esenciales	Contribuyen a la síntesis de anticuerpos y en la resistencia frente algunas enfermedades como la Taurina que se comporta como un aminoácido esencial para recién nacidos y ancianos, siendo la carne una fuente importante para su aporte.
Creatina	Compuesto nitrogenado sintetizado a partir de proteínas (principalmente de carne y pescado), posee un importante efecto en el metabolismo energético del músculo, ya que ciertos suplementos de este compuesto incrementan su rendimiento.
Glutatión	Péptido con efecto reductor, cuya fuente más importante es la carne. Proporciona protección celular frente a una amplia variedad de procesos toxicológicos y patológicos, por lo que puede ser importante en la prevención frente a enfermedades crónicas. Asimismo se le considera como el posible responsable del efecto atribuido a la carne de aumentar la absorción de hierro no hemo de otros alimentos.
L-Carnitina	Péptido que aporta energía y disminuye los niveles de colesterol, ayudando en la absorción del calcio. Procede en un 75% de la dieta, principalmente por la carne roja. Además, posee capacidad antioxidante y puede ser crítico en el desarrollo normal del cerebro. Asimismo disminuye la aparición de ácido láctico y acelera el quemado de grasas al aumentar la función mitocondrial.
Carnosina y anserina	Péptidos bioactivos con capacidad antioxidante, más abundantes en la carne, protegen frente al estrés oxidativo, tiene propiedades antiinflamatorias y antitumorales.
Péptidos antihipertensivos	Péptidos bioactivos que inhiben la enzima angiotensina-I convertasa, con el consiguiente efecto sobre la presión sanguínea. Principalmente son derivados de proteínas de la leche, pero algunos se obtienen a partir de hidrolizados proteicos de músculo, como miopentapeptidos A y B, hidrolizados de miosina de cerdo.

La presencia de ácidos grasos monoinsaturados (AGMI) llega a alcanzar en torno al 44-50% del total en carne de vacuno, porcino, ovino y aves (Tabla I.5), con proporciones de ácido oleico por encima del 40%. Estos compuestos presentan efectos hipocolesteromiantes, disminuyendo los niveles de colesterol asociado a lipoproteínas de baja densidad (*low-density lipoproteins*, LDL), sin alterar los de alta densidad (*high-density lipoproteins,* HDL) (Mattson & Grundy, 1985). Los lípidos de la carne contienen una importante concentración de ácidos grasos poliinsaturados (AGPI), de los cuales el ácido linoleico es el principal componente. Los AGPI pueden representar del 7 al 25% del total de ácidos grasos (Tabla I.5). La presencia en la dieta de AGPI provoca

la disminución de los niveles de LDL-colesterol en el plasma, si bien a diferencia de los AGMI, también reduce la de HDL-colesterol (Mattson & Grundy, 1985). Dadas las evidencias del efecto beneficioso de los AGPI *n-3* de cadena larga EPA [ácido eicosapentaenoico] y DHA [ácido docosahexaenoico] en las enfermedades cardiovasculares (ECV), se ha autorizado el uso de la declaración de las propiedades saludables de los ácidos grasos *n-3* para reducir el riesgo de ECV en alimentos convencionales que contuvieran determinados niveles de EPA y DHA (FDA, 2004; Jacobsen et al., 2008). Un aspecto a destacar es la presencia de cantidades variables de ácido linoleico conjugado (CLA) (0,6-14,9 mg/g de grasa) en la carne, al cual se le han atribuido entre otros, efectos anticancerígenos y antiaterogénicos (Higgs, 2000).

Tabla I.8. Ingesta (%) de los compuestos lipídicos proporcionados por la carne y productos cárnicos en la dieta de la población de algunos países europeos (Hulshof et al., 1999)

Países	**Grasa total**	**AGS**	**AGMI**	**AGPI**	***trans***
Bélgica	26.7	25.1	32.2	19.4	20.7
Finlandia	23.0	19.5	28.5	23.0	4.8
Francia	23.4	20.2	30.0	19.1	11.4
Alemania	28.9	18.2	43.1	21.9	5.3
Grecia	11.4	13.9	10.9	8.9	14.6
Islandia	17.6	18.9	21.4	8.4	15.0
Italia	13.3	15.3	12.6	6.9	13.4
Países bajos	21.2	20.1	29.5	12.9	12.3
Noruega	18.6	18.6	27.1	7.9	6.6
Portugal	28.3	28.1	30.8	19.8	26.2
España	23.7	29.0	24.2	15.8	29.8
Suecia	19.3	17.6	25.5	15.3	10.1
Inglaterra	18.5	17.1	19.2	12.2	10.3

AGS: acidos grasos saturados; AGMI: acidos grasos monoinsaturados; AGPI: acidos grasos poliinsaturados; *trans*: acidos grasos *trans*

La carne y los productos cárnicos presentan niveles de colesterol que en general se sitúan entre 60-75 mg/100 g. Valores que sin embargo, pueden considerarse relativamente bajos comparados con los de algunos otros alimentos, si se exceptúan los existentes en determinados órganos (p.e. hígado). Se ha estimado que en general, el consumo de carne y sus derivados aporta de la mitad a un tercio de los niveles diarios de ingestión recomendados (300 mg) (Chizzolini et al., 1999). De los 257 mg de colesterol/día ingeridos por el consumidor medio en USA en los años 1992-1993, menos del 30% fue de origen cárnico (Schweitzer, 1995). En España de los 440 mg/día,

hasta el 75% es aportado conjuntamente por la carne (40%) (Tabla I.6) y los huevos (35%) (Varela et al., 1996). Las concentraciones elevadas de colesterol sérico y LDL-colesterol se consideran factores de riesgo de las ECV, así como los niveles bajos de colesterol HDL. En general, según algunos autores el colesterol de la dieta aumenta el colesterol LDL y total (Beynen & Katan, 1985; Howell et al., 1997). Sin embargo, este aumento esta más condicionado por la ingesta de grasa saturada y *trans* que por el colesterol dietético. Por otra parte, este aumento de colesterol dietético puede ser especialmente marcado en personas con alteraciones o infecciones hepáticas debido a la elevación del colesterol hepático (Tribble et al., 2008).

I.2.1.3. Vitaminas y minerales

La carne y sus derivados son una fuente importante de diversos minerales y vitaminas, esenciales para el organismo humano (Tabla I.5).

La carne y sus derivados son una excelente fuente de vitaminas del grupo B (Tabla I.6): tiamina (B_1), riboflavina (B_2), niacina, ácido pantoténico, vitamina B_6 y vitamina B_{12}. No contienen cantidades importantes de vitaminas A, C, D, E y K, aunque abundan en algunos órganos (hígado, riñón, etc.). Contribuyen notablemente al aporte de vitaminas a la dieta, de estos alimentos procede el 18% de la tiamina ingerida, el 15% de riboflavina, el 19% de vitamina B_6, el 51% de vitamina B_{12} (Schweitzer, 1995) y el 20% de vitamina D (Higgs, 2000). Se ha señalado que las vitaminas B_6 y B_{12} previenen del riesgo de enfermedades cardiovasculares, al actuar como cofactores de enzimas que disminuyen los niveles de homocisteína en la sangre, compuesto que constituye un factor de riesgo de ECV (McKinley et al., 2001; Wagemakers et al., 2009).

La carne es una buena fuente de hierro, cinc y fósforo, con cantidades significativas de otros elementos traza esenciales como selenio y magnesio (Tabla I.5 y 6). Alrededor de dos tercios del hierro presente está en forma hemo, que se absorbe mejor que el hierro no hemínico existente en alimentos de origen vegetal. Incluso aunque en valor absoluto el nivel de hierro pueda parecer bajo en ciertos tipos de carne como por ejemplo en el pollo, comparado con algunos productos vegetales, su elevada biodisponibilidad hace que su contribución relativa en la dieta sea muy notable (Tabla I.6). Además, la carne favorece la absorción del hierro no hemínico procedente de otros alimentos. Alrededor del 14-22% del hierro de la dieta procede del consumo de carne y sus derivados (Schweitzer, 1995; Varela et al., 1996; Higgs, 2000). La carne es la fuente alimentaria más rica de Zn, también con alta biodisponibilidad (20-40%) (Higgs, 2000)

y en selenio, que es uno de los mejores antioxidantes considerados en la protección contra enfermedades coronarias y cáncer (Higgs, 2000).

Mientras que la carne como tal es relativamente pobre en sodio, ya que contiene solamente entre 50-90 mg por 100 g (Romans et al., 1994), los derivados cárnicos presentan niveles muy superiores proporcionados por la sal añadida, hasta el 2% en productos tratados por el calor (p.e. salchichas) y hasta el 6% en productos crudos curados, en los que la deshidratación hace aumentar aún más su proporción. Las estimaciones realizadas teniendo en consideración los hábitos alimenticios indican que aproximadamente entre un 20-30% de la ingestión de sodio común provendría del consumo de carne y de sus derivados (Wirth, 1991). El sodio dietético es uno de los principales factores asociados con la hipertensión, presentando una relación directa con la presión sanguínea (WHO/FAO, 2003). El efecto del exceso de ingesta de Na sobre la hipertensión se basa en la incapacidad del riñón de eliminar su exceso, aumentando así la presión arterial. En los últimos años, los productos cárnicos han reducido su contenido en sal debido tanto a la conveniencia de limitar la presencia de sodio, como a la menor dependencia de su efecto conservador. Esto contribuiría a la reducción de la ingesta de sodio y de este modo a la prevención de la hipertensión especialmente en personas de alto riesgo. Esto supone una estrategia complementaria a la utilización de antihipertensivos (Kotchen & McCarron, 1998).

I.2.1.4. Otros compuestos de interés

La carne y productos cárnicos, como otros alimentos complejos, experimentan importantes cambios químicos durante los diversos procesos de elaboración y etapas de comercialización (picado, cocción, conservación, exposición a la luz, etc.). Como consecuencia de tales cambios se origina la formación de numerosos compuestos, muchos de los cuales contribuyen a impartir características deseables en el alimento. Sin embargo, algunos otros pueden tener propiedades biológicas indeseables, potencialmente tóxicas para la salud, aunque no es fácil establecer sus consecuencias (Tabla I.9). Entre los compuestos capaces de inducir efectos adversos para la salud se encuentran los hidrocarburos aromáticos policíclicos, las nitrosaminas, los productos de oxidación de los lípidos, las aminas biógenas, etc. (Jiménez-Colmenero et al., 2001a).

Tabla I.9. Estrategias y posibilidades de mejorar las concentraciones de compuestos fisiológicamente activos en la carne y productos cárnicos (Jiménez-Colmenero et al., 2012)

Modificación de la composición de la carne (Tejido animal) con estrategias genéticas y nutricionales:
- Reducción del contenido en grasa. - Mejora del perfil de ácidos grasos: reducción de los AGS, aumento de los AGMI, AGPI, CLA, y la mejora de la relación AGPI/AGS y n-6/n-3. - Reducción del contenido en colesterol. - Incremento del nivel de minerales (selenio, hierro…). - Incremento de antioxidantes (vitamina E, vitamina C, flavonoides…).
Reformulación de productos cárnicos:
- Reducción del contenido en grasa y calorías. - Mejora del perfil de ácidos grasos (reemplazando la grasa animal por la grasa vegetal y/o aceites de origen marino): reducción del contenido de AGS y *trans* y incremento de los AGMI, AGPI (ALA, EPA, DHA), CLA, y mejora de las relaciones AGPI/AGS y n-6/n-3. - Reducción del contenido en colesterol. - Mejora de la calidad de aminoácidos (proteínas no cárnicas). - Incorporación de prebióticos, probióticos y simbióticos. - Enriquecimiento con minerales (selenio, calcio…). - Incorporación de vitaminas y antioxidantes (ácido fólico, tocoferol, carotenoides…). - Reducción del contenido en sodio, nitritos y fosfatos. - Eliminación de alérgenos. - Incorporación de otros ingredientes buenos para la salud (esteroles de plantas, lecitina…).
Condiciones del procesamiento de la carne, de su almacenamiento y su consumo:
- Minimizar el impacto de estas etapas en términos de perdida del contenido en los compuestos bioactivos y su biodisponibilidad. - Promover la formación de compuestos beneficiosos para la salud (péptidos bioactivos, CLA…). - Minimizar la formación de compuestos negativos para la salud (PAH, HCA, aminas biógenas, nitrosaminas y los productos de oxidación lipídica).

Nota: ALA: ácido α-linolénico; CLA, ácidos linoleico conjugado; DHA, ácido docosahexaenoico; EPA, ácido eicosapentaenoico; HCA, amino heterocíclico; AGMI, ácidos grasos monoinsaturados; PAH, hidrocarbonos policíclicos aromáticos; AGPI, ácidos grasos poliinsaturados; AGS, ácidos grasos saturados; *trans*: ácidos grasos *trans*

Algunos compuestos nitrogenados no proteicos se ha descrito que presentan efectos beneficiosos (Tabla I.7). Sin embargo, también existen otros compuestos nitrogenados que pueden tener efectos negativos sobre la salud. De estos compuestos podemos notificar algunas aminas biógenas, como la tiramina e histamina, cuyos efectos tóxicos se manifiestan cuando alcanzan cantidades elevadas en el organismo humano (apartado I.1.3.1).

Diversos compuestos mutagénicos como los hidrocarburos aromáticos policíclicos (*polycyclic aromatic hidrocarbons*, PAH), aminas heterocíclicas y N-nitrosaminas se pueden formar en la carne cocida a altas temperaturas (Bingham et al., 2002; Alaejos et al., 2008). Estos compuestos pueden inducir la formación de aductos de ADN, provocando daños y mutaciones en el mismo (Potter, 1999). Los nitratos y nitritos se incorporan en la elaboración de algunos productos cárnicos ya que inhiben el crecimiento microbiano, fundamentalmente de las bacterias anaerobias (especialmente *Costridium botulinum*), además retardan el desarrollo de la rancidez por su capacidad antioxidante, estabilizan el color y contribuyen a otros aspectos sensoriales. Los nitratos y nitritos a temperaturas elevadas interaccionan con las aminas secundarias (cadaverina y putrescina), dando lugar a la formación de nitrosaminas (previamente mencionado en el apartado I.1.3.1) que poseen propiedades cancerígenas, principalmente sobre el tracto intestinal. Es por ello que el informe del World Cancer Research Foundation (WCRF, 1997) apunta que los nitratos y nitritos deben ser considerados carcinógenos por humanos ya que pueden ser convertidos a compuestos N-nitrosos. La formación de estos compuestos se ve afectada por otros componentes dietéticos, incluyendo el hierro hemo, el cual puede catalizar la reacción de formación de los compuestos N-nitrosos (Santarelli et al., 2008). Los ácidos grasos poliinsaturados y el colesterol pueden experimentar fenómenos de oxidación durante los procesos de elaboración y conservación de la carne y productos cárnicos. Tales fenómenos originan la aparición de numerosos compuestos (hidroperóxidos, aldehidos, cetonas, óxidos del colesterol, etc.), a algunos de los cuales se le atribuye también efectos mutagénicos, cancerígenos y propiedades citotóxicas (Decker & Xu, 1998).

I.3. ESTRATEGIAS PARA MEJORAR LA COMPOSICIÓN DE LA CARNE Y SUS DERIVADOS

De todo lo expuesto se desprende que como en cualquier otro alimento, en la carne y productos cárnicos existen diversos elementos que, en determinadas circunstancias y en proporciones inadecuadas, pueden afectar negativamente la salud humana (Jiménez-Colmenero, 2001a). En consecuencia la posibilidad de obtener carne y derivados cárnicos más saludables pasa por aplicar procedimientos apropiados para optimizar su composición, esto se logra reduciendo o eliminando compuestos con efectos negativos para la salud y favoreciendo la presencia de aquellos otros con implicaciones positivas. Las estrategias empleadas para llevar a cabo tal tarea pueden ser fundamentalmente de tres tipos: a nivel de producción animal, asociadas a la transformación de materias primas cárnicas y a través de los procesos de reformulación de derivados cárnicos (Jiménez-Colmenero, 1996). Las oportunidades (tecnológicas o biotecnológicas) que permiten tales estrategias (Tabla I.9) se basan en los siguientes aspectos (Jiménez-Colmenero, 2005; Jiménez-Colmenero et al., 2012):

- **Limitar** la presencia de un componente con efectos negativos. Tales compuestos pueden estar naturalmente presentes en el alimento o ser formados durante su procesado, conservación, etc. (Ejemplo: reducción de ácidos grasos saturados, ácidos grasos *trans*, compuestos tóxicos o alergenos…)
- **Incrementar** la concentración de un compuesto (nutriente o no), naturalmente presente en el alimento, con efectos beneficiosos. Este incremento puede ser naturalmente inducido (Ejemplo: aumentar la presencia de determinados componentes en la canal, y por lo tanto en los cortes comerciales mediante estrategias de producción animal, caso de ácido graso *n-3*, ácido linoleico conjugado, vitamina E) o producido mediante procesos de reformulación (Ejemplo: enriquecimiento con calcio en derivados cárnicos o lácteos).
- **Reemplazar** un componente, generalmente un macronutriente, cuya ingestión es habitualmente excesiva y por ello causa efectos negativos por otro con efectos beneficiosos (Ejemplo: modificar el perfil de ácidos grasos, reemplazar saturados por insaturados, mejorar la relación n-6/n-3).
- **Adicionar** un componente con efectos beneficiosos que normalmente no se encuentra presente en el alimento (Ejemplo: fibra de productos cárnicos).

- **Modificar** la naturaleza de uno o más componentes con el propósito de mejorar los efectos beneficiosos para la salud (Ejemplo: proteínas hidrolizadas para reducir la probabilidad de reacciones alérgicas o la producción de péptidos bioactivos).
- **Mejorar** la biodisponibilidad (Ejemplo: aumentar la absorción) o la estabilidad de un componente para preservar su efecto funcional.
- Hacer **combinaciones** de las posibilidades descritas anteriormente.

Dependiendo del tipo de producto (constituido por trozos de carne identificables, picado grosero o muy fino, gel/emulsión, aplicación de tratamientos térmicos, curado, etc.), una de las mayores oportunidades de modificar la composición de los alimentos se deriva de la posibilidad de actuar en alguna de las etapas de elaboración y, en la medida de lo posible, proceder a su reformulación con el propósito de desarrollar una gama de derivados de composición y propiedades especificas diseñadas a conveniencia. El desarrollo de este tipo de productos exige tener en cuenta numerosos aspectos por cuanto el nuevo elaborado cárnico ha de responder de manera adecuada en relación con propiedades tecnológicas, sensoriales y nutricionales, seguridad, conveniencia, etc., por otro lado similares a los exigidos a cualquier producto de naturaleza análoga (Jiménez-Colmenero, 2000).

Por sus implicaciones en la salud (Jiménez-Colmenero, 2001a), tanto los lípidos como el contenido en sodio son los compuestos que han recibido una mayor atención. La mejora del contenido lipídico de los derivados cárnicos se ha llevado a cabo fundamentalmente a dos niveles: **reducción del contenido en grasa** y/o **modificación del perfil de ácidos grasos**, reduciendo la presencia de AGS y favoreciendo la de AGMI y AGPI, en especial los de cadena larga. Mientras que la mejora del contenido en sodio se ha llevado a cabo a nivel de **reducción del contenido de sal,** sustituyendo la sal añadida por una mezcla de sales que no contienen Na.

I.3.1. Mejora del contenido lipídico: reducción de grasa animal

La reducción del contenido en grasa, por cuanto su presencia condiciona de manera fundamental las características sensoriales del producto, no es una tarea fácil que pueda llevarse a cabo empleando simplemente menos grasa en la formulación. La posibilidad de desarrollar productos cárnicos con un contenido lipídico óptimo va a depender de varios factores como son el nivel de grasa deseado, la naturaleza del producto a reformular (sistemas gel/emulsión, características estructurales como grado de desintegración, coexistencia de estructuras con diferente granulometría, untuosidad, etc.) y el tipo de

procesado requerido por el mismo (formación de la emulsión, tratamientos térmicos, maduración, entre otros) (Jiménez-Colmenero, 2001b).

La elaboración de productos cárnicos con menor nivel de grasa generalmente responde a dos criterios básicos, la utilización de materias primas cárnicas más magras (lo que encarecería el costo de la formulación) y la disminución de la densidad de grasa y calorías mediante la adición de agua y otros ingredientes con escasa o nula aportación de calorías. Además, se puede requerir el empleo de determinados procedimientos que coadyuven a compensar las modificaciones que se inducen al variar la composición y naturaleza del producto. Todo ello se puede abordar a través de la utilización de manera individual o conjunta de diversos procedimientos que, haciendo posible la reducción del nivel y/o modificación de las características de la grasa presente, permita la obtención de un producto que exhiba unas condiciones de funcionalidad, seguridad, propiedades sensoriales y estabilidad adecuadas. Tales procedimientos están basados en la: 1) selección de ingredientes cárnicos de manera que se disponga de una materia prima conveniente tanto desde el punto de vista de su composición como de su aptitud tecnológica, 2) utilización de ingredientes no cárnicos capaces de impartir textura y en especial a favorecer la capacidad de retención de agua, y 3) adecuación de la tecnología de elaboración y/o preparación a la conveniencia de inducir ciertas características funcionales en el producto final (Jiménez-Colmenero, 2001b).

Las sustancias empleadas como sustitutivos de grasa han de suponer por una parte, una aportación escasa de calorías, y por otra contribuir a impartir al producto las características deseadas. Su potencial aplicación es fundamentalmente en productos en donde existe cierta desintegración estructural de las materias primas y por lo tanto es posible el íntimo contacto entre los diversos constituyentes. La mayoría de los ingredientes y/o aditivos empleados para disminuir el nivel de grasa se pueden categorizar como: 1) Agua añadida; 2) Proteínas de origen no cárnico (soja, surimi, proteínas de origen lácteo, harina de trigo, albúminas, etc.); 3) Carbohidratos (gomas o hidrocoloides, almidones y maltodextrinas y derivados de la celulosa), y 4) Otros productos (mezclas funcionales, aceites vegetales, productos sintéticos).

I.3.1.1. Sustitutos de origen proteico

Derivados proteicos tanto de origen animal como vegetal, han sido utilizados en la elaboración de productos cárnicos para incrementar el rendimiento (propiedades ligantes de agua y grasa), rebajar costos de formulación, potenciar propiedades funcionales

específicas (capacidad de retención de agua, propiedades emulsionantes) y reducir el contenido en grasa.

Varios autores han indicado la posibilidad de disminuir la cantidad de grasa total, ácidos grasos saturados (AGS) y ácidos grasos *trans* mediante la incorporación de sustitutos de grasa como proteínas de soja, maíz, huevo, trigo, etc. (Pietrasik & Duda, 2000; Gujral et al., 2002). Entre las proteínas de origen no cárnico más utilizadas se encuentran las de origen vegetal (especialmente de soja) y de origen animal como la proteína de huevo y de la leche (Dexter et al., 1993; Pietrasik & Duda, 2000; Gujral et al., 2002; Serdaroğlu, 2006). En la tabla I.10 se recogen unos ejemplos de sustitutos de grasa animal más usados en los productos cárnicos. Estos componentes además de sus contribuciones nutricionales y tecnológicas, pueden presentar efectos saludables que proporcionan un valor añadido a su empleo. La soja, por ejemplo, presenta efectos beneficiosos en relación con la prevención y el tratamiento de enfermedades cardiovasculares, cáncer y osteoporosis, y en el alivio de los síntomas menopáusicos (Hasler, 1998). Por otra parte, la proteína de girasol rica en L-arginina, y tiene una baja proporción de L-lisina/L-arginina, contribuye a la prevención de la hipercolesterolemia y agregación de plaquetas.

I.3.1.2. Sustitutos de origen lipídico

Son compuestos lipídicos o de naturaleza análoga que presentan propiedades similares a las de la grasa animal y tienen un bajo o nulo aporte calórico en el producto cárnico. Se pueden clasificar en diferentes grupos en función de sus características, añadiéndose al alimento con distintos propósitos (reducir y/o modificar la composición de la grasa). Una primera categoría la constituyen los triglicéridos de diseño, esterificados con ácidos grasos de diferente longitud de cadena, configurados químicamente para que su aporte calórico sea muy reducido o incluso cero. Estos incluyen productos como los denominados “Saltrim” (5 cal/g) y “Caprenin” (5 cal/g). Un segundo grupo lo constituyen compuestos sintéticos basados en grasas modificadas, para reemplazar grasas y aceites. Tienen características organolépticas similares a la grasa animal pero resistentes a las enzimas del estómago, entre ellos esta el “Olestra” (0 cal/g).

Tabla I.10. Ejemplos de sustitutos proteicos para la reducción de grasa animal en productos cárnicos.

Sustitutos proteicos	Productos en los que se ha sustituido	Referencias
Soja	Carne picada	Kotula & Berry, 1986
	Salchichas frescas (búfalo)	Ahmad et al., 2010
	Carne picada (vacuno)	Dignam et al., 1979; Ziprin et al., 1981; Ali et al., 1982; Deliza et al., 2002; Kilic et al, 2010
	Paté de vacuno	Rhee & Smith, 1983; Brewer et al., 1992; Heywood et al., 2002; Kassama et al., 2003; Rentfrow et al., 2004
	Hamburguesa	Zhu et al., 2001; Angor & Abdullah, 2010; Kassem & Emara, 2010
	Salchichas cocidas	Pietrasik & Duda, 2000
	Mortadela de pavo	Dexter et al., 1993
Suero	Albóndigas	Serdaroğlu, 2006
	Paté de vacuno (cocido)	El-magoli et al., 1995; Hale et al., 2002
	Paté de cerdo (cocido)	Andic et al., 2010
	Carne emulsionada	Peña-Ramosa & Xiong, 2003
	Hamburguesa de vacuno	Desmond et al., 1998
Colágeno	Carne picada (vacuno)	Chavez et al., 1986; Rao & Henrickson, 1986; Graves et al., 1994; Campbell et al., 1996
	Reestructurado de vacuno	Kenney et al., 1992
	Salchichas frankfurt	Eilert et al., 1996
Proteína de huevo	Paté de cabra	Gujral et al., 2002

El principal inconveniente del uso de sustitutos de origen lipídico es la posible disminución de la absorción de vitaminas liposolubles, esto debe ser tomado en cuenta en alimentos que son fuentes de vitaminas liposolubles en la dieta (Serrano & Sánchez-González, 2008).

I.3.1.3. Sustitutos basados en carbohidratos

Los carbohidratos que se han empleado en la reformulación de productos cárnicos con bajo nivel de grasa son básicamente fibras, gomas o hidrocoloides de distintas procedencias. En general, su utilización tiene como objetivo de mejorar el rendimiento en la cocción, incrementar la capacidad de retención de agua, reducir los costos de reformulación, modificar la textura y mejorar la estabilidad a la congelación.

Entre los principales tipos de fibra empleados como sustitutos se encuentran la del trigo, derivados de maíz, harina de algodón, avena, diversos tipos de almidón, pectinas, celulosa, maltodextrinas, carragenatos, etc. (Jiménez-Colmenero et al., 2010a) (Tabla I.11). Últimamente, la utilización de algunas fibras solubles está adquiriendo gran importancia como análogos de grasa, entre las que se encuentran la inulina (fibra soluble extraída de la achicoria) y el konjac glucomanano (KG) los cuales se han

incorporado a distintos productos cárnicos (Berry & Bigner, 1996; Mendoza et al., 2001; Kao & Lin, 2006; Jiménez-Colmenero et al., 2010a) (Tabla I.11).

I.3.1.3.1. Konjac Glucomanano (KG)

De entre las distintas fibras conocidas, el konjac glucomanano (KG) resulta de especial interés por sus efectos fisiológicos y sus excepcionales propiedades tecnológicas que le confiere un gran potencial de aplicación en la tecnología de alimentos. Su uso como aditivo alimentario está autorizado en Europa (E-425), habiendo sido reconocido como sustancia GRAS por la Food and Drug Administration (FDA). En los últimos años el interés en este polisacárido ha crecido tanto en Europa, que está siendo analizada la posibilidad de integrar su sistema productivo en el marco de la agricultura Europea (Final report abstract FAIR-CT98-4106).

El konjac glucomanano (KG) es un polisacárido neutro producido por la planta *Amorphophallus konjac* originaria del este de Asia, donde se emplea desde la antigüedad. Esta planta requiere de un procesamiento previo para obtener el glucomanano. El tubérculo de konjac se seca, se tritura, se muele y se purifica para obtener el glucomanano que representa entre un 30-80% del peso (seco) del tubérculo (Tye, 1991; González Canga et al., 2004). El glucomanano obtenido (Figura I.6) es un polisacárido lineal de peso molecular entre 200.000 y 2.000.000 daltons (superior al de cualquier otra fibra conocida), cuya estructura química esta constituida por D-manosa y D-glucosa (en una relación entre 1,4:1 y 1,6:1), unidas por enlace β (1→4). La cadena principal presenta algunos puntos de ramificación, aproximadamente 1 cada 11 residuos, ligados a la posición C-3 de la manosa y generalmente de entre 11-16 residuos de longitud. De igual modo, entre el 5-10% de los residuos de manosa/glucosa de la cadena principal del glucomanano se encuentran acetilados, considerándose que es la presencia de este grupo la que le confiere su solubilidad en el agua (Williams et al., 2000; Huang et al., 2002).

Tabla I.11. Ejemplos de sustitutos de grasa basados en carbohidratos para la reducción de grasa animal en productos cárnicos.

Sustitutos carbohidratos	Productos en los que se ha sustituido	Referencias
Almidón patatas	Paté Coreano tradicional	Muhlisin et al., 2012
	Salchichas de vacuno cocidas	Liu et al., 2008
Almidón de trigo	Paté de vacuno	Rocha-Garza & Zayas, 1995
Harina de sorgo	Paté vacuno cocido	Huang et al., 1999
Almidón de maíz	Carne de vacuno picada	Khalil, 2000
Harina de lentejas	Albóndigas	Serdaroğlu et al., 2005
Harina de garbanzos	Albóndigas	Serdaroğlu et al., 2005
	Croquetas de pollo	Verma et al., 2012
	Carne de vacuno picada	Shaner & Baldwin, 1979
Harina de frijol	Albóndigas	Serdaroğlu et al., 2005
Cebada	Carne de vacuno picada	Bond et al., 2001
Remolacha	Salchichas frankfurt	Vural et al., 2004
Fibra dietética	Carne de vacuno picada	Troutt et al., 1992
	Salchichas frankfurt	Grigelmo-Miguel et al., 1999
Mezcla de fibras*	Paté de vacuno	Tornberg & Sjöholm, 2005
Fibra de guisante	Hamburguesa de vacuno	Anderson & Berry, 2000, 2001; Besbes et al., 2008
Fibra de trigo	Hamburguesa de vacuno	Mansour & Khalil, 1999
	Chorizo	García et al., 2002
Fibra de avena	Hamburguesa de vacuno	Desmond et al., 1998; Troy et al., 1999; Chevance et al., 2000
	Paté de vacuno	Chevance et al., 2000; Pinero et al., 2008
	Salami de vacuno	Chevance et al., 2000
	Salchichas frankfurt	Hughes et al., 1997; Chevance et al., 2000
	Chorizo	García et al., 2002
Fibra de avellana	Hamburguesa de vacuno	Turhan et al., 2005
Fibra de cítrico	Salchichas frankfurt	Cengiz & Gokoglu, 2007
Fibra de soja	Salchichas tipo bologna	Cofrades et al., 2000
Fibra de arroz	Carne emulsionada	Choi et al., 2009, 2010
Fibra de limón	Hamburguesa	Aleson-Carbonell et al., 2005
Fructooligosacáridos	Mortadela (bologna)	Cáceres et al., 2004
Konjac glucomanano	Hamburguesa, salami y salchichas	Patanawongyuneyong, 2002
	Salchichas frankfurt	Jiménez-Colmenero et al., 2010a; Kao & Lin, 2006; Lin & Huang, 2003
	Nuggets de cerdo reestructurados	Berry & Bigner, 1996
	Mortadela	Chin et al., 1998 a, b, 2000
	Salchichas frescas	Osburn & Keeton, 1994
Inulina y fibra altramuz	Salchichas	Archer et al., 2004
Salvado de centeno	Albóndigas	Yilmaz, 2004
Salvado de arroz	Reestructurado de vacuno	Kim et al., 2000
Celulosa	Hamburguesa de vacuno	Hill & Prusa, 1989
	Chorizo	Bastianello Campagnol et al., 2012
Tapioca	Hamburguesa de vacuno	Berry, 1997; Desmond et al., 1998;Troy et al., 1999; Chevance et al., 2000
	Paté, salchichas frankfurt y salami de vacuno	Chevance et al., 2000
	Carne de búfalo picada	Nisar et al., 2009
	Salchichas de vacuno frankfurt	Hughes et al., 1998; Sampaio et al., 2004
Carragenato	Hamburguesa de vacuno	Troy et al., 1999
	Salchichas frankfurt	Hughes et al., 1997; Cierach et al., 2009; Jiménez-Colmenero et al., 2010a
	Salchichas bologna	Ruusunen et al., 2003
	Salchichas cocidas	Pietrasik & Duda, 2000
	Mortadela de pavo	Dexter et al., 1993
Beta-glucano	Paté de vacuno	Warner & Inglett, 1997; Pinero et al., 2008
	Salchichas frescas	Morin et al., 2004
Maltodextrina	Carne de vacuno picada	Garzon et al., 2003
	Salchichas frankfurt	Crehan et al., 2000
Amilopectina	Paté de vacuno	Warner & Inglett, 1997
Polidextrosa	Carne de vacuno picada	Troutt et al., 1992

* Coliflor, brócoli, coles de Bruselas, judías verdes, guisantes, ortigas, tomate, repollo, espinaca seca, nabo de churaco, nabo de espinaca, zanahoria, apio, rábano negro, remolacha, rosa mosqueta, manzana y peras.

I.3.1.3.1.1. Efectos fisiológicos

La harina de konjac se considera un ingrediente escasamente calórico que dado su contenido en fibra no digestible, presenta numerosos efectos fisiológicos y aplicaciones terapéuticas (Tye, 1991; Zhang et al., 2001; González Canga et al., 2004; Al-Ghazzewi et al., 2007), muchas de las cuales han sido aprovechadas tradicionalmente en Japón y China.

Según Kaats et al. (2004), la ingestión del konjac contribuye a reducir el apetito debido a su elevada capacidad de absorción de agua que proporciona sensación de saciedad. Este hecho aumenta la viscosidad del contenido gastrointestinal retrasando el vaciado gástrico y prolongando así la sensación de plenitud. Estos fenómenos conllevan una reducción del peso corporal. También, el konjac se ha mostrado eficaz para el tratamiento de estreñimiento crónico por el incremento en el volumen de heces (Marsicano et al., 1995). Ha sido además, usado para el control dietético de diabetes dada su capacidad de reducir los niveles sanguíneos de glucosa e insulina, probablemente debido a que al retrasar el vaciado gástrico se dificulta el acceso de la glucosa a la mucosa intestinal (González Canga et al., 2004). Varios autores han señalado el efecto hipocolesteromiante del konjac, ya que provoca un descenso del colesterol y de la fracción LDL (Arvill & Bodin, 1995; Martino et al., 2005), así como de triacilglicerol (Takigami, 2000; Kao & Lin, 2006). Este hecho le ha atribuido la propiedad de prevenir enfermedades cardiovasculares (González Canga et al., 2004). El KG ha sido también descrito como prebiótico dado que contiene componentes que estimulan selectivamente el desarrollo de bacterias intestinales beneficiosas (Al-Ghazzewi et al., 2007). También algunos autores han señalado que esta fibra no limita la biodisponibilidad de minerales como el calcio, hierro cobre o zinc (González Canga et al., 2004). El konjac glucomanano juega además un papel activo en la inhibición en la génesis de tumores y metástasis (Gao & Nishinari, 2004). Sin embargo, el konjac tiene algunos inconvenientes como la producción de flatulencias, molestias abdominales, etc. (González Canga et al., 2004).

Figura I.6. Harina de konjac.

I.3.1.3.1.2. Propiedades tecnológicas

El Konjac glucomanano (KG) presenta extraordinarias características físico-químicas que hacen posible su empleo como agente espesante, gelificante o para modificar la textura. El KG es una fibra muy soluble que posee una excepcional capacidad de retención de agua, hasta más de 100 veces su peso, proporcionando una elevada viscosidad a las soluciones que forma (Tye, 1991; González Canga et al., 2004). Esta fibra es capaz de formar películas (muy estables en agua fría y caliente, en medios ácido o básico) y membranas (Tye, 1991; Zhang et al., 2001), con interesantes propiedades gelificantes condicionadas por su peso molecular, contenido en grupos acetilo, presencia de impurezas, medio de gelificación y temperatura a la que se emplea. Al disolverse en medio alcalino (p.e. por adición de hidróxido cálcico o carbonato potásico o sódico) se forman geles térmicamente estables (incluso a 200° C) por liberación de los grupos acetilo, permitiendo a la molécula interaccionar dando lugar a las estructuras poliméricas que constituyen los geles. Zhang et al. (2001) han sugerido que los puntos de asociación de estos geles están constituidos por enlaces de hidrógeno formados entre regiones desacetiladas de la cadena. Sin embargo, para el KG nativo, la gelificación (sin desacetilar) necesita concentraciones elevadas (> 7%) o largos periodos de tiempo (Williams et al., 2000; Penroj et al., 2005).

Existen efectos sinérgicos entre el KG y los almidones e hidrocoloides (kappa carragenato, goma xantana, goma gellan, etc.), esto hace posible la formación de geles con diferentes propiedades físico-químicas (incluidas las de superficie) y por tanto con distintas oportunidades de aplicación en tecnología de alimentos (Tye, 1991; Miyoshi et al., 1996; Yoshimura et al., 1996; Huang & Lin, 2004).

I.3.1.3.1.3. Aplicación como sustituto de grasa en los productos cárnicos

Una posible manera de sustituir la grasa en derivados cárnicos es el empleo de geles de konjac como ha sido descrito anteriormente. Las características de dichos geles dependen de factores asociados a la cantidad y naturaleza de los ingredientes, pH (alcalino) y condiciones de procesado, entre ellas la temperatura (Berry & Bigner, 1996; Osburn & Keeton, 1994, 2004). Los geles de KG son capaces de simular las propiedades organolépticas de la grasa (sensación en la boca) y tejido conectivo en sistemas cárnicos (Tye, 1991). El konjac combinado con almidón (2-4%) y algunas gomas (carragenatos, goma gellan-0,25-0,50%, etc.) ha sido usado en la reducción de grasa animal de productos como salchichas tipo "frankfurt" (Lin & Huang, 2003; Osburn & Keeton, 2004; Kao & Lin, 2006), mortadela (Chin et al., 1998 a, b, 2000), salchichas frescas (Osburn & Keeton, 1994) o nuggets de cerdo (Berry & Bigner, 1996) (Tabla I.11). El efecto de incorporación de geles de KG varía en función del tipo de producto cárnico, cambio deseado de la composición (reducción calórica/grasa, relación humedad/proteína), contenido y características del polisacárido empleado, presencia de fenómenos de sinergismo y condiciones de procesado del derivado cárnico. Distintos autores han usado geles de KG en combinación con otros ingredientes (almidón, carragenatos, goma gellan) como "análogos de grasa" (Tye, 1991; Berry & Bigner, 1996; Lin & Huang, 2003; Osburn & Keeton, 2004; Kao & Lin, 2006) para la reformulación de productos cárnicos con bajo contenido calórico. Tales análogos son "troceados" dependiendo del producto cárnico reformulado, presentando así apariencia, propiedades tecnológicas y sensoriales similares a los de la grasa animal (Tye, 1991). Una posibilidad interesante a considerar, es la combinación del konjac con aceites, que además de reducir grasa favorecería una mejora en el perfil de ácidos grasos (aceite de oliva por ejemplo) en la matriz cárnica.

A pesar de la evidente potencialidad que ofrece el KG en el desarrollo de "análogos de grasa", por cuanto además de hacer posible la reducción del nivel de grasa, presenta por si mismo adicionales implicaciones beneficiosas para la salud. Sin embargo, hasta donde se conoce, no se ha llevado a cabo estudios sobre su incorporación en productos crudos curados, tipo chorizo, y frescos, tipo merguez.

I.3.2. Mejora del contenido lipídico: modificación del perfil de ácidos grasos

La composición de los ácidos grasos presentes en los productos cárnicos puede ser modificada (de manera simultánea o no a la reducción de grasa), básicamente mediante dos procedimientos. El primero de ellos se sitúa a nivel de producción animal (utilización de estrategias genéticas y de alimentación). Partiendo de materias primas cárnicas ricas en ácidos grasos insaturados (AGI) procedentes de animales alimentados con grasas vegetales (cártamo, girasol y canola), se han elaborado productos que obviamente también presentan mayor proporción de estos ácidos grasos (St. John et al., 1986; Shackelford et al., 1990). El segundo procedimiento consiste en reemplazar, en mayor o menor medida, la grasa animal habitualmente presente en el producto por otra de características más convenientes para la salud, por tanto con menor proporción de ácidos grasos saturados y mayor de monoinsaturados (oleico) o poliinsaturados, y además sin colesterol. La simple sustitución de la grasa, si bien no supone una disminución del contenido en calorías, implica una mejora importante en el perfil lipídico del producto. Con tal propósito se han empleado tanto aceites de pescado (ricos en *n-3* poliinsaturado) como vegetales (ricos en AGMI y AGPI) en muchos tipos de productos cárnicos (Tabla I.12). Entre estos tipos de aceites, cabe destacar el aceite de oliva por su contenido en AGMI y elevado valor biológico (López-López et al., 2010a) (Tabla I.12). También cabe reseñar la combinación de aceites (vegetales y de pescado) con el objetivo de reducir las relaciones AGS/AGMI y n-6/n-3 en los productos cárnicos reformulados (Delgado-Pando et al., 2010 a, b, 2011 a, b, 2012).

I.3.2.1. Aceite de oliva

El aceite de oliva es el aceite vegetal con mayor contenido de ácidos grasos monoinsaturados que se conoce, contiene 56-87% de AGMI, 8-25% AGS (IOOC, 1984) y 4-22% de AGPI. Este aceite tiene un alto valor biológico atribuido a su alto contenido en antioxidantes naturales (vitamina E, K, carotenoides y varios polifenoles) y una relación baja de AGS/AGMI comparado con otros aceites vegetales. Es una fuente de, por lo menos, 30 compuestos fenólicos (Tuck & Hayball, 2002), entre ellos hidroxitirosol, tirosol y oleuropeina, los cuales actúan como antioxidantes (Boskov, 1996; Leenen et al., 2002). La potencial influencia positiva del aceite de oliva en la salud incluye la prevención de los riesgos de enfermedades cardiovasculares y protección contra el deterioro cognitivo, la enfermedad de Alzheimer y el desarrollo de

cáncer de colón y de mama (López-Miranda et al., 2006; Waterman & Lockwood, 2007).

Por lo tanto, podría ser interesante incorporar el aceite de oliva en los productos cárnicos reformulados como estrategia para la mejora del perfil lipídico de los mismos. En este sentido se han realizado diversos estudios para incorporar este aceite vegetal en diferentes productos cárnicos (Tabla I.12). Sin embargo en la literatura científica no existen muchos trabajos sobre productos cárnicos frescos y ninguno indicando su empleo combinado con konjac glucomanano (Tabla I.12).

I.3.2.2. Combinación de aceites de origen vegetal y marino

Es evidente que la adición de lípidos individuales de origen vegetal (p.e. aceite de oliva) o marino mejora el perfil de ácidos grasos de los productos cárnicos reformulados, sin embargo, una aproximación más acertada para lograr un perfil lipídico óptimo desde un punto de vista saludable, puede alcanzarse de manera más conveniente usando una combinación de aceites como sustituto de grasa animal. Es por ello que una gran cantidad de autores han desarrollado este tipo de estrategia para mejorar el perfil de ácidos grasos en los productos cárnicos (Paneras et al., 1998; Delgado-Pando et al., 2010b; García-Iñiguez de Ciriano et al., 2010). De estos estudios cabe destacar los realizados por Delgado-Pando et al. (2010a). Estos autores han logrado formando una emulsión hecha con una mezcla de aceites como sustituto de grasa de cerdo e incorporarla en varios productos cárnicos. Tal combinación está formada por aceites vegetales (de oliva y de lino) y de pescado en proporciones y niveles adecuados para proporcionar un perfil de ácidos grasos ajustado a los objetivos de ingesta saludable. Se trata de un material lipídico con proporciones reducidas de AGS y elevadas cantidades de AGMI y AGPI (incluyendo los *n-3* de cadena larga) y una relación balanceada de n-6/n-3 AGPI y AGPI/AGS (Delgado-Pando et al., 2010b). Esta mezcla se ha integrado en un gel de konjac glucomanano y se ha adicionado a pâté (Delgado-Pando et al., 2011, 2012). Sin embargo, no existen estudios sobre la incorporación de esta mezcla (junto con konjac) en embutidos crudos curados tipo chorizo. Por lo tanto, sería interesante estudiar este enfoque para mejorar el perfil lipídico de este tipo de productos cárnicos.

I.3.3. Reducción del contenido en sodio

El sodio es uno de los factores más implicados en la hipertensión. Su disminución en productos cárnicos se ha investigado durante décadas (Gelabert et al., 2003). Sin embargo, el empeño no es fácil ya que la sal es esencial en la elaboración de productos cárnicos, debido a la importante función que ejerce sobre la textura, sabor y desarrollo microbiano (Weiss et al., 2010). Es por ello que reducciones de sal llevan asociadas la aparición de problemas tecnológicos, sensoriales y microbiológicos. A fin de compensar los efectos asociados a una menor presencia de NaCl, se han utilizado distintos sustitutos, entre los cuales el KCl ha sido el más empleado, si bien su concentración se ha limitado a 0,5-0,6%, ya que a niveles superiores aparecen sabores amargos y metálicos (Desmond, 2006). Una combinación de sales de sodio, potasio, magnesio y calcio ha sido particularmente investigada en diferentes tipos de productos cárnicos dando resultados satisfactorios (Frye et al., 1986; Lin et al., 1991; Collins, 1997; Gimeno et al., 1998, 1999, 2000; Armenteros et al., 2009; Aliño et al., 2010; Ripollés et al., 2011). También se han incorporado compuestos como fosfatos y sales de ácidos orgánicos. Coadyuvando el papel de los distintos tipos de sales, se emplean otros ingredientes y aditivos, como proteínas, fibra dietética, hidrocoloides y almidones, para mejorar la estabilidad de los productos por su capacidad de retención de agua y grasa (Gimeno et al., 2001; Ruusunen et al, 2003; Ruusunen & Poulane, 2005; Ruiz-Capillas & Jiménez-Colmenero, 2009; Totosaus & Pérez-chabela, 2009; Verma et al., 2010).

I.4. PRODUCTOS CÁRNICOS FRESCOS Y CRUDOS CURADOS

La carne y productos cárnicos han sido tradicionalmente muy apreciados por el ser humano, entre ellos ocupan un papel destacado los embutidos crudos curados y salchichas frescas. Estos son preparados a partir de carnes picadas sometidas a procesos de curación, adicionadas de despojos comestibles o grasas, productos vegetales, condimentos y especias, e introducidos en tripas naturales o artificiales (Luzón Merino & Bejarano, 2001). Según el Código Alimentario Español, capítulo X, Carnes y Derivados, se definen como embutidos y longanizas crudos/as aquellos que han sido sometidos únicamente al adobo y amasado antes del llenado en tripa, fermentados o no y sometidos posteriormente al secado y ahumado o no. No contendrán tejido fibroso, cartílagos ni sebos. Básicamente existen a) Los embutidos crudos curados entre los que se encuentra el chorizo que es un producto muy apreciado por los consumidores en los

países europeos y especialmente en España y b) Las longanizas tipo salchichas frescas, entre las cuales se puede incluir el merguez que es uno de los productos cárnicos más apreciados en los países del Norte de África (donde se consumen y se fabrican principalmente) y en algunos países Europeos.

I.4.1. Productos crudos curados: chorizo

La fermentación de la carne es un proceso antiguo originalmente utilizado para prolongar la vida útil de materias primas perecederas. Durante la fermentación suceden numerosas reacciones bioquímicas complejas y procesos físicos que originan un cambio significativo de las características iniciales. Además, la producción de sustancias aromáticas durante la fermentación define las características sensoriales del producto final que son diferentes de las materias primas utilizadas.

Los embutidos crudos curados son un grupo de productos tradicionales mediterráneos con una gran diversidad entre países e incluso entre regiones de un mismo país (Aymerich et al., 2003). Su tecnología de fabricación varía en toda Europa. La fermentación se hace en condiciones controladas durante un período de tiempo, de manera que el producto desarrolla poco a poco unas características de color, sabor y textura. Los diversos tipos de embutidos fermentados se elaboran con mezclas de especias, cultivos iniciadores y distintos tipos de materias primas. Existe una gran variedad de chorizos con denominaciones que a menudo hacen referencia a su región de origen, como por ejemplo chorizo de Pamplona. Estos chorizos son generalmente elaborados de carne picada o troceada de cerdo y tocino y/o grasas de cerdo, adición de sal, pimentón y otras especias, condimentos y aditivos autorizados, amasada y embutida en tripas naturales o artificiales. Son sometidos a un proceso de maduración-secado, con o sin ahumado, y se caracterizan por su color rojo (con excepción de los denominados chorizos blancos) debido al pimentón añadido y por su sabor característico (Luzón Merino & Bejarano, 2001). En España, para que un embutido sea denominado chorizo, ha de llevar necesariamente pimentón y ajo; esto lo diferencia de otros países.

Tabla I.12. Ejemplos de ingredientes lipídicos para la mejora del perfil de ácidos grasos en productos cárnicos.

Sustitutos lipídicos	Forma de incorporación	Productos en los que se sustituyó	Referencias
Oliva	Pre-emulsión/CS[a]; Interesterificado	Salchichas frankfurt	Bloukas & Paneras, 1993; Paneras & Bloukas, 1994; Pappa et al., 2000; Vural et al., 2004; Choi et al., 2010; Jiménez-Colmenero et al., 2010b; Herrero et al., 2012; Álvarez et al., 2012
	Pre-emulsión	Carne emulsionada	Choi et al., 2009
		Paté (hígado de cerdo)	Martín et al., 2008
	Líquido: pre-mezclado	Salami	Severini et al., 2003
	Pre-emulsión/CS[a]	Chorizo pamplona	Muguerza et al., 2001
	Pre-emulsión/APS[b]	Hamburguesa	López-López et al., 2010b; Rodríguez-Carpena et al., 2012
	Pre-emulsión	Chorizo	Bloukas et al., 1997; Muguerza et al., 2002
	Pre-emulsión	Turkish soudjouk (sucuk)	Kayaardi & Gök, 2003; Koutsopoulos et al., 2008
Girasol	Pre-emulsión/CS[a]	Salchicha frankfurt	Paneras & Bloukas, 1994
	Líquido	Salchicha frankfurt y salami cocido	Ambrosiadis et al., 1996
Girasol alto oleico	Líquido	Salchicha frankfurt	Park et al., 1989, 1990
Canola	Pre-emulsión	Salchichas frankfurt	Choi et al., 2010; Álvarez et al., 2011, 2012
		Carne emulsionada	Choi et al., 2009
	Pre-emulsion/APS[b]	Salchichas fermentadas alemanas	Pelser et al., 2007
		Carne de vacuno picada	Garzon et al., 2003; Youssef & Barbut, 2011
Maíz	Pre-emulsión/CS[a]	Salchichas frankfurt	Paneras & Bloukas, 1994; Choi et al., 2010
	Pre-emulsión/CS[a]	Mortadela Bologna	Bishop et al., 1993
	Pre-emulsión	Carne emulsionada	Choi et al., 2009
	Sólido: PH[c]; Liquido	Hamburguesa	Dzudie et al., 2004
	Líquido	Salchicha frankfurt y salami cocido	Ambrosiadis et al., 1996
Soja	Sólido: PH[c]	Hamburguesa	Liu et al., 1991
	Líquido	Hamburguesa; Paté; salchichas frankfurt; Salami	Shiota et al., 1995; Ambrosiadis et al., 1996; Hong et al., 2004; Choi et al., 2010
	Pre-emulsión	Carne emulsionada	Choi et al., 2009
	Pre-emulsión/APS[b]	Chorizo Pamplona	Muguerza et al., 2003
	Pre-emulsión/CS[a]	Salchichas frankfurt	Paneras & Bloukas, 1994
Semilla de uva	Pre-emulsión	Carne emulsionada	Choi et al., 2009
CLA[d]	Pre-emulsión	Paté (hígado de cerdo)	Martín et al., 2008
Semilla de algodón	Interesterificado	Salchichas frankfurt; Producto semi-curado	Vural et al., 2004; Özvural & Vural, 2008
	Líquido	Salchicha frankfurt y salami cocido	Ambrosiadis et al., 1996
	Sólido: PH[c]	Producto tipo hamburguesa	Liu et al., 1991
Cacahuete	Sólido : PH[c]	Producto tipo hamburguesa	Liu et al., 1991
	Líquido	Salchicha frankfurt	Marquez et al., 1989
Nuez	Sólido	Salchichas frankfurt	Jiménez-Colmenero et al., 2005; Özvural & Vural, 2008
		Salchichas turcas fermentadas (Sucuk)	Yildiz-Turp & Serdaroglu, 2008
Lino	Pre-emulsión/APS[b]	Salchichas fermentadas alemanas	Pelser et al., 2007
		Chorizo Pamplona	Ansorena & Astiasarán, 2004
Aceites esenciales de frutos secos	Líquido	Producto tipo hamburguesa	Dzudie et al., 2004
Pescado	Desodorizado y Líquido Encapsulado	Salchichas frankfurt	Park et al., 1989
		Salchichas fermentadas alemanas	Pelser et al., 2007
	pre-emulsión/APS[b]	Chorizo	Muguerza et al., 2004; Valencia et al., 2006
Palma y derivados	Sólido: PH[c]	Hamburguesa	Liu et al., 1991
	Sólido	Hamburguesa	Shiota et al., 1995
		Hamburguesa de ternera	Babji et al., 2001; Wan Rosli et al., 2006
	Interesterificado	Salchicha frankfurt	Vural et al., 2004
	Fundido (55ºC)	Salchichas frankfurt de pollo	Tan et al., 2006
	Líquido	Producto tipo hamburguesa	Shiota et al., 1995
"Palmine"	Sólido	Salchicha frankfurt y salami cocido	Ambrosiadis et al., 1996
Alga	Pre-emulsión	Chorizo Pamplona	Valencia et al., 2007
	Emulsión de aceite en agua-APSu[e]	Hamburguesa de pavo; Salchichas frescas de cerdo; Jamón reestructurado	Lee et al., 2006 a, b

[a] CS-caseinato sódico; [b] APS-aislado de proteína de soja; [c] PH-parcialmente hidrogenado; [d] CLA-acido linoleico conjugado; [e] APSu-aislado de proteína de suero.

Sin embargo, el chorizo, al igual que otros productos cárnicos, presenta algunas limitaciones debido al alto nivel de grasa (ácidos grasos saturados) componente que se ha señalado como un factor de riesgo en relación con las enfermedades cardiovasculares. Por eso, en los últimos años se han realizado diversos esfuerzos para modificar la composición de este tipo de productos cárnicos a través de estrategias tecnológicas. Tales modificaciones consisten tanto en una reducción de la grasa animal, como en producir una mejora en el perfil lipídico.

I.4.1.1. Reformulación del chorizo

Como en otros productos cárnicos con similares características, los procesos de reformulación del chorizo se han encaminado a reducir el contenido de grasa y mejorar el perfil de ácidos grasos (Muguerza et al., 2004; Jiménez-Colmenero, 2007). Sin embargo, los embutidos fermentados son uno de los productos cárnicos que mayores dificultades presenta a nivel de reducción de grasa. Además de su aporte nutricional, la grasa contribuye a las propiedades de calidad y aceptabilidad (sabor, textura, sensación en la boca, etc.) del chorizo. Por otro lado, la grasa visible granulada también tiene una función tecnológica ya que facilita la liberación regular de humedad que ocurre durante el proceso de fermentación (Wirth, 1988). Diferentes estudios han explorado la posibilidad de reducción de la grasa, usando estrategias de reformulación donde la grasa es reemplazada por carne magra (Mendoza et al., 2001; Muguerza et al., 2002; Liaros et al., 2009; Olivares et al., 2010). En algunos casos esta estrategia está acompañada por la adición de otros ingredientes como la inulina (Mendoza et al., 2001), cereales y fibra de frutas (García et al., 2002) y fructooligosacáridos de cadena corta (Salazar et al., 2009) para reducir su contenido energético y contribuir a impartir las características propias del producto. Sin embargo, generalmente la reformulación aumenta la dureza del producto debido a la mayor pérdida de agua durante la fermentación (Muguerza et al., 2002; Salazar et al., 2009; Olivares et al., 2010). Importantes esfuerzos también han sido realizados para mejorar el perfil de ácidos grasos en el chorizo mediante la incorporación de aceites vegetales o de pescado (Ansorena & Astiasaran, 2004; Koutsopoulos et al. 2008; García-Iñiguez de Ciriano et al., 2009, 2010; Beriain et al., 2011). Uno de los principales problemas tecnológicos que presenta esta estrategia es conseguir una buena estabilización del aceite en la matriz cárnica. Para ello, los procedimientos empleados, cuando se realiza en productos cárnicos, abarcan desde su adición directa en forma de líquido o de sólido, hasta su incorporación en forma

encapsulada, pre-emulsionado, o formando parte de ingredientes vegetales. El procedimiento más adecuado en cada caso depende del tipo de producto, material lipídico, y naturaleza y magnitud de la modificación propuesta, entre otros.

I.4.1.2. Presencia de aminas biógenas en chorizo

Las condiciones que acontecen durante los procesos de fermentación, maduración y almacenamiento del chorizo (temperatura de maduración alta, pH ácido, alta disponibilidad de aminoácidos libres, presencia de enzimas microbianas con actividad aminoácido descarboxilasa, larga maduración, etc.) pueden favorecer la descarboxilación bacteriana de aminoácidos libres e inducir la acumulación de las aminas biógenas en el producto final (Suzzi & Gardini, 2003; Vidal-Carou et al., 2007). En general, muchos autores han señalado la existencia de altas proporciones de aminas biógenas en el chorizo, siendo las más representativas, tiramina, putrescina y cadaverina, junto con las aminas fisiológicas, espermidina y espermina, que son específicas de la carne y sus derivados (Tabla I.13 y I.14). Las citadas condiciones de elaboración y conservación junto con algunos factores que se modifican en la reformulación del producto pueden variar la cantidad y el perfil de aminas biógenas en estos productos (Tabla I.13 y I.14).

Teniendo en cuenta que las aminas biógenas tienen efectos toxicológicos (sólos o asociados a otros compuestos) sobre la salud cuando alcanzan ciertas cantidades, resulta necesario conocer su producción a lo largo de las distintas etapas de procesado, fermentación, maduración y almacenamiento. Es por ello que, a pesar de la ausencia de normas específicas y reglamentos relativos a su aparición en embutidos fermentados, hay un creciente interés en estos compuestos. No existen estudios que evalúen el efecto de la reformulación del chorizo (reducción o sustitución de la grasa animal y mejora del perfil lipídico), así como durante su procesamiento, maduración y almacenamiento sobre la producción de aminas biógenas.

I.4.2. Salchichas frescas: merguez

El merguez es una salchicha fresca elaborada con carne roja originaria de los países norteafricanos que tiene un alto grado de aceptación en muchos países de Europa. Los ingredientes, características de materias primas y variables de procesamiento utilizados en su elaboración condicionan la calidad de este producto popular consumido en numerosos países del mundo (El Ayachi et al., 2007).

Tabla I.13. Ejemplos de niveles de aminas biógenas (mg/kg) formados durante el procesado del chorizo.

		Aminas biógenas									
Variable considerada		**Tyr**	**His**	**Pea**	**Put**	**Cad**	**Tryp**	**Agm**	**Spd**	**Spm**	**Referencia**
Sin starters		ND-77	ND-0.5	ND	ND-7	ND-45	ND	-	6.8-8.1	45.4-77.9	Bover-Cid et al., 2000a
Starters (*L. sakei* + *S. carnosus*)		ND-7	ND-0.5	ND	ND-3	ND-3	ND	-	6.6-7.3	59.6-85.9	
Starters (*L. sakei* + *S. xylosus*)		ND-10	ND-0.5	ND	ND-3	ND-2	ND	-	6.4-7.5	62.1-84	
Carne no envasada y refrigerada 5 días		40-360	5.3-7.9	5.6-6.4	ND-75	40-260	16.8-21.4	1.2-32	-	-	Bover-Cid et al., 2000b
Carne envasada y congelada 5 días		ND-110	ND-1.4	ND	ND-10	ND-20	1-2.6	0.8-1.8	-	-	
Sin sulfitos		ND-80	ND	ND	1.02-7.38	ND-46	ND	1.37-4.64	7-8	44-65	Bover-Cid et al., 2001a
Con sulfitos	500 – 1000 mg/kg	ND-140	ND	ND	0.92-12.33	0-2	ND	1.25-2.49	7-8	44	
Sin starters		ND-84	<0.4	<1	ND-5	ND-23	<1	-	5-7	47-55	Bover-Cid et al., 2001b
Starters (*L. curvatus* y *S. xylosus*)		ND-80	<0.4	ND	ND-2	ND-2	ND	-	5-7	47-55	
Carne refrigerada		ND-80	<1	ND	ND-7	ND-30	ND	-	-	-	Bover-Cid et al., 2006
Carne congelada		ND-93	<1	ND	ND-23	ND-4	ND	-	-	-	
Sin starters		ND-80	<0.5	ND	ND-34	ND-1.76	ND-1.39	ND	4.0-5.6	47.9-41.6	Bover-Cid et al., 1999b
Starters (*Staphylococcus spp.*)		ND-58	<0.5	ND-0.5	ND-32	ND-1.36	ND-1.19	ND	5.6	41.6	
Sin starters		62-132	ND	ND-5	78-223	ND-19	ND-50	-	ND-4	ND-2	González-Fernández et al., 2003
Starter (*Pediococcus sp. P22*)		8-85	ND	ND-1	4-252	ND-10	ND-16	-	ND-4	ND-11	
Starter (*Pediococcus sp. P208*)		12-102	ND	ND-2	ND-231	ND-19	ND-35	-	ND-5	ND-9	
Sin starters	nP	ND-55	ND	ND	ND-32	ND-80	ND	-	6-18	56-88	Latorre-Moratalla et al., 2007
	P	ND-58	ND	ND	ND-6	ND-1	ND	-	8-10	58-66	
Starters (*L. sakei* + *S. xylosus*)	nP	ND-10	ND	ND	ND-4	ND-4	ND	-	7-10	67-75	
	P	ND-10	ND	ND	ND-4	ND-1	ND	-	9-11	58-62	
Sin starters		ND-200	ND	-	ND-220	ND-280	-	-	-	-	Latorre-Moratalla et al., 2010b
Starter: *L. sakei*		ND-160	ND	-	ND-160	ND-70	-	-	-	-	
Starter: *S. equorum*		ND-225	ND	-	ND-250	ND-150	-	-	-	-	
Starter: *L. sakei* + *S. equorum*		ND-170	ND	-	ND-170	ND-25	-	-	-	-	
Proceso industrial Starters*: Pediococcus acidilactici* + *Staphylococcus vitulus*		57.2-233	12.4-361.7	-	ND	2.1-812.5	36.7-143.8	-	0.5-7.3	149.8-297.7	Casquete et al., 2012

Tyr: Tiramina; His: Histamina; Pea: β-feniletilamina; Put: Putrescina; Cad; Cadaverina; Tryp: Triptamin; Agm; Agmatina; Spd: Espermidina; Spm: Espermina; P: Tratamiento con presión hidrostática (200MPa/10min/17ºC); nP: Tratamiento sin presión hidrostática; ND: no detectado

Tabla I.14. Ejemplos de niveles de aminas biógenas (mg/kg) formados a lo largo de la conservación del chorizo.

		Aminas biógenas									
Variable considerada	**Conservación**	**Tyr**	**His**	**Pea**	**Put**	**Cad**	**Tryp**	**Agm**	**Spd**	**Spm**	**Referencia**
Elaborado con carne R+NE	4 ºC	360-420	7.9	6.4	75-110	260-300	21.4	32	-	-	Bover-Cid et al., 2000b
	15 ºC	360-500	7.9	6.4	75-150	260-375	21.4	32	-	-	
Elaborado con carne C+E	4 ºC	110-115	1.4	ND	10-14	20-22	2.6	1.8	-	-	
	15 ºC	110-145	1.4	ND	10-20	20-25	2.6	1.8	-	-	
Sin starters	4 ºC	82-115	ND	<1.5	5	22-25	<1.5	-	6.6	45.3	Bover-Cid et al., 2001b
	19 ºC	82-116	ND	<1.5	4	22-30	<1.5		5.7	38.2	
Starters (*L. curvatus* y *S. xylosus*)	4 ºC	30-90	ND	<1.5	1-3	1-2	<1.5	-	6.6	45.3	
	19 ºC	42-190	ND	<1.5	1-2	2-3	<1.5		5.7	38.2	
Proceso tradicional		29.2-626.8	ND-314.3	ND-51.5	2.6-415.6	ND-658.1	ND-87.8	-	1.4-10.0	13.8-43.5	Hernández-Jover et al., 1996b, 1997
NE		214.45	15.53	-	185.14	229.33	-	2.08	8.15	39.94	Ruiz-Capillas & Jiménez-Colmenero, 2004
ATM		153.21	0.53	-	88.57	70.83	-	0.93	7.57	58.83	
P		18.51	0.94	-	0.87	9.02	-	43.02	6.74	39.10	
Proceso industrial pH < 5.5		10-180	ND-190	ND-75	ND-260	2-35	ND-85	-	1.22-4.83	8.27-33.67	Miguélez-Arrizado et al., 2006
Proceso tradicional pH > 5.5		ND-110	ND	ND-75	ND-100	ND-120	ND-85	-	1.55-6.71	9.71-55.12	
nP		92.125	0.2-1.7	1-17	148-238	3.5-8.5	-	0.5	3.5-6	24.34	Ruiz-Capillas et al., 2007c
P		88-104	0.6-1.6	1-15	136-220	3.5-7.4	-	0.5-1.2	3.5-5.5	25-31	

Tyr: Tiramina; His: Histamina; Pea: β-feniletilamina; Put: Putrescina; Cad; Cadaverina; Tryp: Triptamin; Agm; Agmatina; Spd: Espermidina; Spm: Espermina.
R+NE: Refrigerada + No envasada; C+E: Congelada + Envasada; nP: Tratamiento sin alta presión; P: Tratamiento con alta presión; ATM: Tratamiento con atmosfera modificada; ND: no detectado

Se elabora con carne de cordero, ternera o una mezcla de ambas, embutida en una tripa de cordero, que se dividen para formar unidades más cortas, con dimensiones que varían en función de las demandas del mercado. A continuación, las salchichas se secan a temperatura ambiente, o se mantienen en refrigeración durante un corto periodo de tiempo, para su posterior almacenamiento en condiciones de refrigeración (2–4 ºC). Estas salchichas están fuertemente condimentadas con una amplia variedad de especias como pimentón, pimienta, harissa que es una pasta de guindilla que le da su característico sabor picante y color rojo. Otros ingredientes que se añaden son hinojo y ajo. El merguez suele consumirse a la parrilla, cocido o secado al sol.

Como cualquier otro producto cárnico fresco, el merguez resulta más perecedero que otros tipos de derivados cárnicos, se deteriora rápidamente debido a la alta actividad microbiana situándose la vida media de almacenamiento, en condiciones de refrigeración, en torno a 3-5 días (Benkerroum et al., 2003; El Ayachi et al., 2007). Para prolongar el periodo de conservación de este tipo de productos, se han empleado varias estrategias, entre ellas la incorporación de bacteriocinas (Scannell et al., 2000; Benkerroum et al., 2003), de mezclas comerciales de lactato y acetato sódico (El Ayachi et al., 2007) y de sulfitos (Banks & Board, 1982; Scannell et al., 2000).

El merguez, al igual que el chorizo (apartado I.4.1), presenta también un alto contenido en grasa animal (23%) y sodio (891 mg/100g) (ANSES, 2008; Tabla I.5). Mejorar la composición de este producto pasa por la aplicación de estrategias tecnológicas encaminadas a la reducción de grasa animal, la mejora del perfil lipídico y la reducción de sodio.

I.4.2.1. Reformulación de salchichas frescas

En salchichas frescas no se conocen estudios en relación con la mejora de su perfil lipídico, y es muy escasa la información sobre procesos de reformulación a nivel de reducción de grasa. Osburn & Keeton (1994) elaboraron salchichas frescas con el objetivo de reducir grasa (animal) incorporando el konjac glucomanano en su formulación. Con igual propósito, Ahmad et al. (2010) reemplazaron grasa animal por proteína de soja en salchichas frescas de búfalo.

Recientemente, como se ha comentado anteriormente (apartado I.3.3), se ha recomendado una reducción de la ingesta de sal a la luz de la relación entre los niveles altos de sodio y la hipertensión arterial. Un gran porcentaje de la población mundial posee predisposición genética a la hipertensión arterial, que se ve afectada por exceso de

peso y la alta ingesta de sodio. En el marco de la estrategia NAOS (2009) se ha publicado recientemente un informe analizando las principales fuentes de sodio en la dieta, entre las que los embutidos ocupan el primer lugar (Tabla I.15). La reducción de sodio se realiza sustituyendo parcialmente el cloruro de sodio añadido por otros compuestos que deberán aportar efectos similares a nivel de propiedades sensoriales, tecnológicas y microbiológicas (apartado I.3.3). Dependiendo del tipo de producto, estas sustituciones presentan distintas dificultades (Wirth, 1991). La carne como tal es relativamente pobre en sodio, contiene menos de 100 mg de Na por 100 g, en contra posición a sus derivados (Ruusunen & Puolanne, 2005). Las estimaciones indican que aproximadamente el 30% de la ingesta de sodio común proviene de la carne y productos cárnicos (Wirth, 1991; NAOS, 2009; Tabla I.15). Como en el caso del contenido en lípidos, existen pocos estudios sobre la reducción de sodio en los productos tipo merguez (Pasin et al., 1989). Sin embargo, notables esfuerzos han sido llevados a cabo para reducir el contenido en Na en productos cárnicos crudos curados o cocidos (Collins, 1997; Ruusunen & Puolanne, 2005; Desmond, 2006). La mejor manera de bajar el nivel de sodio en productos cárnicos, como se ha señalado anteriormente (apartado I.3.3), sería la sustitución del NaCl, con otras sales (potasio, calcio y magnesio). Aunque la sustitución total del NaCl parece difícil de conseguir por razones sensoriales y tecnológicas, una combinación de sales de sodio, potasio, magnesio, calcio puede producir resultados más satisfactorios (Frye et al., 1986; Lin et al., 1991; Collins, 1997; Gimeno et al., 1998, 1999, 2000; Armenteros et al., 2009; Aliño et al., 2010; Zanardi et al., 2010; Ripollés et al., 2011).

Otro aspecto a considerar en el caso del merguez, hace referencia a su limitada estabilidad y presencia de las aminas biógenas.

I.4.2.2. Presencia de aminas biógenas en salchichas frescas

No se conocen estudios sobre la formación de aminas biógenas en salchichas frescas tipo merguez, aunque si en carne y productos cárnicos frescos. Dichos estudios, como ya se ha comentado, indican que la producción de aminas biógenas puede estar condicionada por múltiples factores tales como la calidad de la materia prima usada (carne, grasa, ingredientes), las condiciones de procesamiento (higiene de los aparatos usados para el procesamiento, temperatura de la masa, etc.) y las condiciones de conservación (temperatura de almacenamiento, tipo de embalaje, etc.). Se han descrito numerosos estudios acerca de la formación de aminas biógenas y de los factores que

intervienen en su producción en salchichas frescas. La mayoría de trabajos están dirigidos a aumentar la estabilidad del producto limitando el desarrollo microbiano, y en consecuencia la presencia de aminas biógenas (Krizek et al., 1995; Kaniou et al., 2001; Lee & Yoon, 2001; Rokka et al., 2004; Cannarsi et al., 2005; Saccani et al., 2005; Patsias et al., 2006; Ruiz-Capillas & Jiménez-Colmenero, 2010; Curiel et al., 2011). Sin embargo, hasta donde se conoce no existen estudios sobre los niveles de aminas biógenas en salchichas frescas reformuladas para mejorar el contenido lipídico y reducir el nivel de sodio.

Tabla I.15. Principales fuentes de sodio y su porcentaje en la ingesta diaria (NAOS, 2009)

Productos	**% sodio total**	**Sal/100g PC**	**G PC/día**
Embutidos	26,16	2,90	56,7
Pan y panes especiales	19,06	1,10	109
Leche y lácteos	15,60	0,22	436
Pescados y derivados	7,23	0,58	77,6
Platos preparados	4,85	1,02	29,9
Carnes frescas	4,01	0,21	117
Sopas y cremas	4,01	0,32	77,9
Salsas	3,24	1,28	14,3
Verduras y hortalizas	3,01	0,06	297
Bollería	2,20	0,64	21,5
Huevos y derivados	1,69	0,36	29,4
Azúcares y dulces	1,60	0,41	24,6
Aceitunas y variantes	1,46	2,32	3,9
Otros cereales	1,99	0,23	59,1
Galletas	1,25	0,54	14,5
Aperitivos salados	1,03	1,09	5,9
Bebidas	0,85	0,01	1025
Frutas y derivados	0,27	0,01	251
Mantequillas y margarinas	0,23	0,23	4,5
Legumbres y derivados	0,16	0,04	26,2
Condimentos	0,11	0,12	5,9

PC: Porción comestible

II. OBJETIVOS

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En base a todo lo expuesto, **el objetivo general de la presente memoria consiste en desarrollar procesos de reformulación de derivados cárnicos encaminados a obtener productos más saludables y estudiar como tales procesos condicionan la formación de aminas biógenas**. El estudio abarca dos tipos de elaborados cárnicos con características y condiciones de procesado diferentes como son chorizo, como ejemplo de embutido crudo curado, y merguez como modelo de productos frescos típicos (étnico) de gran relevancia en distintos países norteafricanos y europeos. Los procesos de reformulación planteados están dirigidos a incidir en el contenido lipídico (reduciendo la presencia de grasa y mejorando el perfil de ácidos grasos) y/o limitar el contenido en sodio.

Este objetivo general se ha abordado a través de los siguientes objetivos específicos:

1. **Desarrollar un procedimiento mejorado para la determinación de aminas biógenas en productos cárnicos**. El objetivo se ha centrado en establecer las condiciones metodológicas óptimas que permitan la cuantificación simultánea de nueve aminas biógenas (tiramina, histamina, β-feniletilamina, putrescina, cadaverina, triptamina, agmatina, espermidina y espermina). Esto es especialmente relevante en el caso de β-feniletilamina y triptamina, aminas presentes en matrices cárnicas y con limitaciones a nivel de determinación en otros procedimientos. Este objetivo trata de resolver las limitaciones metodológicas asociadas a la determinación simultánea de las aminas biógenas.

2. **Ensayar procesos de reformulación en un producto crudo curado (chorizo) encaminados a mejorar su contenido lipídico y analizar como estos procesos condicionan la formación de aminas biógenas.** En este marco el objetivo ha consistido en la aplicación de procesos de reformulación encaminados a la reducción de grasa y/o mejora del perfil de ácidos grasos. Para tal fin, se realizó la sustitución de la grasa animal, habitualmente empleada, por un análogo de grasa basado en un gel de konjac o por una matriz de konjac conteniendo una combinación de aceites de origen vegetal (oliva y linaza) y marino (pescado). La combinación de aceites empleada fue

específicamente establecida para dotar al producto, de un perfil lipídico más ajustado en relación con las recomendaciones nutricionales. En tales circunstancias se ha evaluado la formación de aminas biógenas en función de factores de composición, procesado y conservación. Todo ello encaminado a la obtención de derivados cárnicos más saludables con adecuada viabilidad tecnológica, sensorial y microbiológica.

3. **Estudiar estrategias de reformulación en una salchicha fresca tipo merguez encaminadas a mejorar su contenido lipídico y reducir la presencia de sodio y valorar como estas estrategias condicionan la formación de aminas biógenas**. Este objetivo se ha centrado en el desarrollo de procesos de reformulación dirigidos a la mejora del contenido lipídico basados en la sustitución de la grasa animal, habitualmente empleada, por un análogo de grasa basado en gel de konjac o por una matriz de konjac conteniendo aceite de oliva. De igual modo se ha ensayado la reduccion del contenido en sodio mediante la sustitución de NaCl por una combinación de sales (cloruro de potasio, calcio y magnesio). En tales circunstancias se ha evaluado la formación de aminas biógenas en función de factores de composición y condiciones de conservación. Todo ello encaminado a la obtención de derivados cárnicos más saludables con adecuada viabilidad tecnológica, sensorial y microbiológica.

III. MATERIALES Y MÉTODOS

III. MATERIALES Y MÉTODOS

En este apartado se exponen de manera resumida, con el fin de evitar repeticiones, los materiales y métodos empleados para la realización de los trabajos experimentales descritos en el apartado IV. La figura III. 1 recoge de forma esquematizada el desarrollo experimental abordado y la metodología empleada en esta memoria.

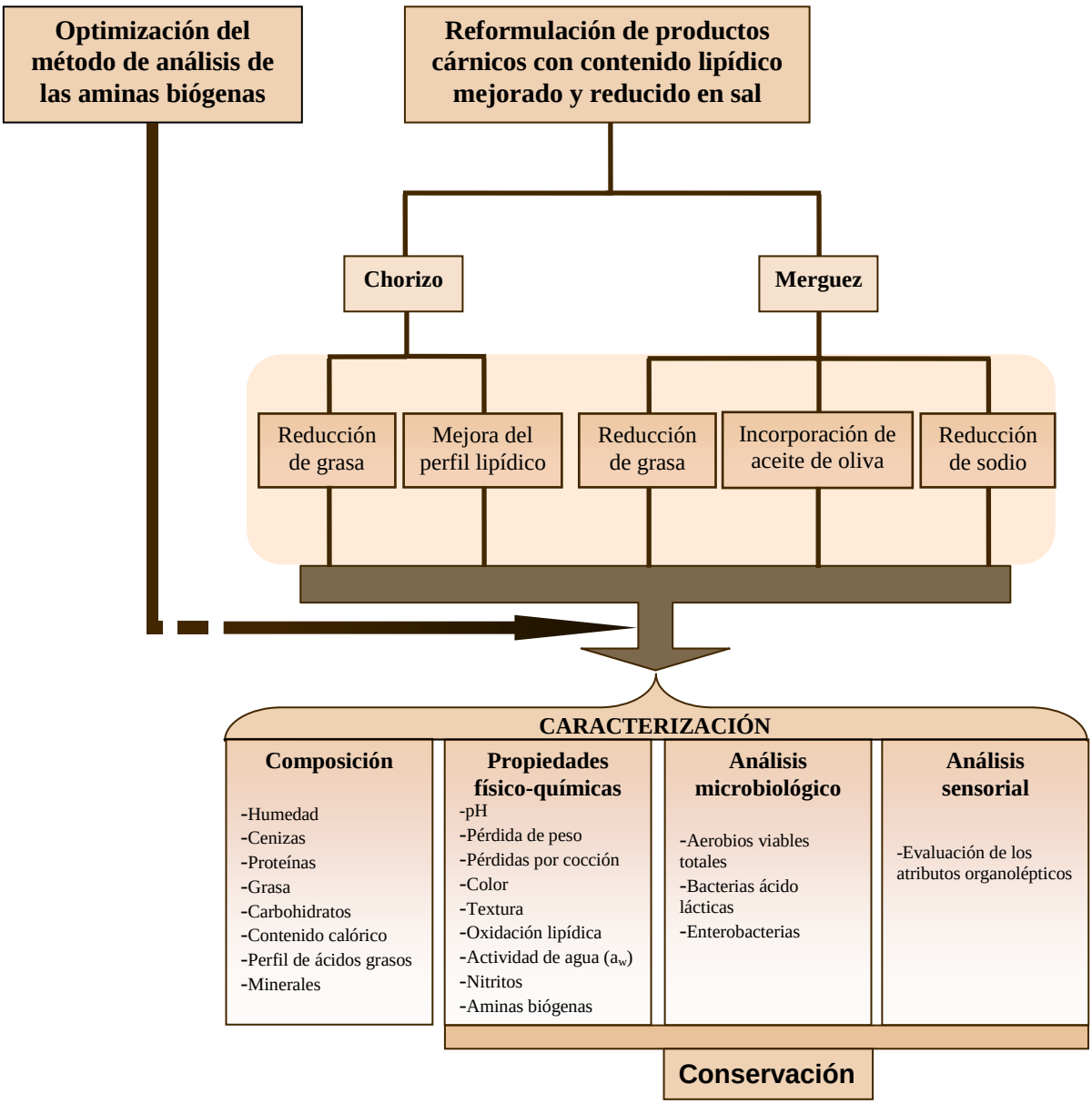

Figura III.1. Esquema general del desarrollo experimental y la metodología, llevados a cabo en esta memoria

III.1. OPTIMIZACIÓN DEL MÉTODO DE DETERMINACIÓN DE AMINAS BIÓGENAS EN PRODUCTOS CÁRNICOS

En este apartado se exponen los diferentes materiales y métodos usados para la optimización del procedimiento de determinación de las aminas biógenas en productos cárnicos.

III.1.1. Reactivos

Se emplearon los siguientes reactivos: ácido tricloroacético (TCA) (Panreac, Barcelona, España), metanol, 2-propanol para HPLC (High Performance Liquid Chromatography), fosfato potásico dibásico, hidróxido potásico, cloruro potásico, ácido acético y solución Brij al 35%, todos estos reactivos fueron suministrados por Sigma-Aldrich (España). El agua ultra pura se obtuvo del sistema Milli-Q (millipore, integral del ICTAN). O-phthalaldehydo (OPA, ref. O120), tiofluor, grado cromatográfico (N, N-Dimetil-2-mercaptoetilamina-hidrocloruro), diluyente de OPA (ref. OD104: compuesto por 3% hidróxido potásico, 3% ácido bórico, 94% agua, pH = 10,40). Las fases móviles empleadas fueron: fase A que consiste en un tampón de fosfato potásico (ref K600: 11% 2-propanol, 0,9% fosfato potásico dibásico, 0,3% ácido acético, 87,8% agua, pH = 6,00), fase B (ref K563: 5% cloruro potásico, 4% 2-porpanol, 0,9% fosfato potásico dibásico, 0,3% ácido acético, 89,8% agua, pH = 5,63) y la fase C potásica de regeneración de la columna (ref: K130: 0,7% cloruro potásico, 4% 2-propanol, 0,5% hidróxido potásico, 94,8% agua, pH = 13,00). Todos los reactivos de las fases móviles fueron suministrados por Pickering (Ca, USA). El o-phtalaldehydo (OPA) que se emplea como reactivo de derivatización post-columna (Pickering Laboratories, Ca. USA) fue preparado con 975 mL de la solución OPA (OD 104), 0,100 mg de OPA disuelto en 10 mL de metanol, 2 g de tiofluor y 3 mL de solución Brij al 35%.

Los patrones empleados para la determinación de aminas biógenas fueron: clorhidrato de tiramina (Tyr), diclorhidrato de histamina (His), clorhidrato de β-feniletilamina (Pea), diclorhidrato de putrescina (Put), diclorhidrato de cadaverina (Cad), triptamina cristalina (Tryp), sal de sulfato de agmatina (Agm), triclorohidrato de espermidina (Spd), tetraclorohidrato de espermina (Spm). Todo ellos adquiridos en Sigma-Aldrich (España).

III.1.2. Preparación de las soluciones estándar

La preparación de las soluciones estándar se realizó a partir de una solución madre de 1000 mg/L de cada amina biógena preparada con TCA al 7,5%. A partir de cada una de estas soluciones se elaboró una solución intermedia de 100 mg/L en TCA 7,5%, conteniendo todas las aminas biógenas. A partir de la cual se prepararon soluciones de trabajo de 0,05 a 12 mg/L de las aminas biógenas también en TCA 7,5% que se conservaron en refrigeración hasta su uso. Las soluciones de trabajo fueron filtradas a través de un filtro Nylon de 0,22 μm de diámetro (Teknokroma, España) y se colocaban en frascos de ámbar de 2 mL con tapón de rosca (PTFE/silicona) (Perkin Elmer, Life and Analytical Sciences, USA) que se disponían en el inyector automático del cromatógrafo.

III.1.3. Extracción de las aminas biógenas

Las muestras de carne y productos cárnicos seleccionadas para el estudio de la optimización del método **(capítulo IV.1.1)** fueron carne fresca de cerdo (*Longuissimus dorsi*) y dos productos cárnicos: uno crudo curado "chorizo" y otro, salchicha tipo frankfurt, adquiridos en un mercado local (Figura III.2). Las muestras fueron elegidas por ser representativas de diferentes matrices complejas y con varios sistemas de procesamiento y producción, y por lo tanto con diferentes niveles de aminas biógenas.

Las aminas biógenas fueron determinadas en un extracto preparado (1:2 p/v) con 15 gramos de muestra en 30 mL de TCA al 7,5% que se homogeneizó en un omnimixer (Omni International, Waterbury, Ct, USA) a 20000 rpm, 1 min. La mezcla se centrifugó a 3800 g durante 15 min a 4 ºC (Sorvall RTB6000B, DuPont, USA). El sobrenadante se filtró a través de un filtro Whatman de 0,45 μm para su uso inmediato, o para conservarla a -20 ºC para su uso posterior. Previo a su inyección en el cromatógrafo (Pickering Laboratories, Ca, USA) todas las muestras se filtraron a través de un filtro de Nylon (Teknokroma, España) de 0,22 μm de diámetro y se colocaron en un vial de ámbar (Perkin Elmer, Life and Analytical Sciences, USA) que se disponía en el inyector automático del cromatógrafo. Los resultados fueron un promedio de al menos 3 determinaciones.

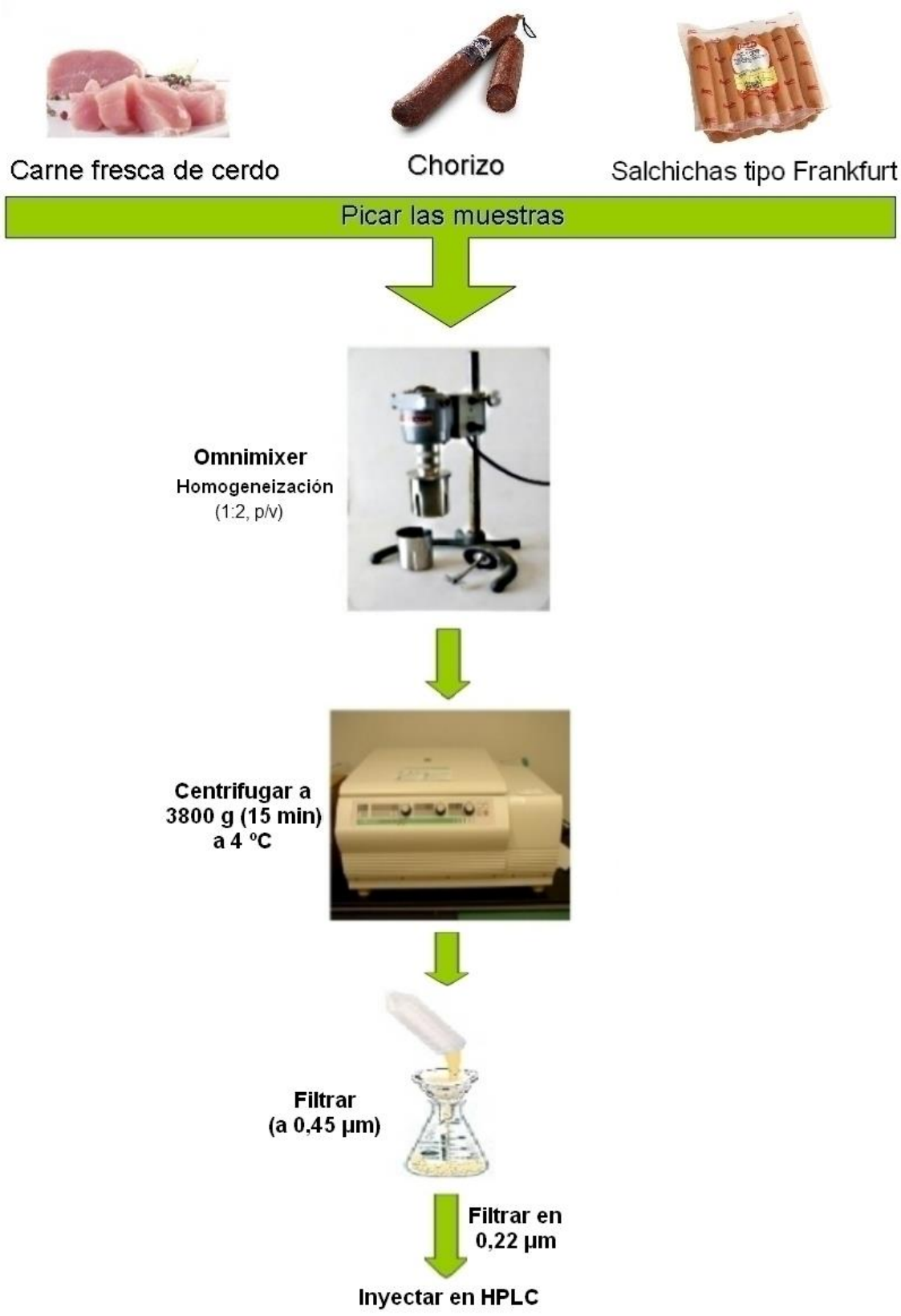

Figura III.2. Procedimiento de extracción de aminas biógenas en carne y productos cárnicos

III.1.4. Determinación del pH de las fases móviles

El pH de las fases móviles se determinó directamente en las fases usando un pH metro (radiómetro PHM 93, Copenhague, Dinamarca).

III.1.5. Análisis cromatográfico (HPLC) de las aminas biógenas

La determinación cromatográfica de aminas biógenas se realizó utilizando un equipo compuesto de una bomba cuaternaria (serie 200, Perkin Elmer, Life and Analytical Sciences, USA), un inyector automático (serie 200, Perkin Elmer, Life and Analytical Sciences, USA), un sistema de post-columna de Pickering PCX 3100 (Pickering Laboratories, Ca, USA) que contiene una columna de intercambio catiónico (K^+, 4 mm x 150 mm) y una pre-columna (K^+, 3 mm x 20 mm), ambas con un tamaño de partícula de 10 μm de diámetro (Pickering Laboratories, Ca, USA). Las condiciones de partida del programa de elución de acuerdo a Ruiz-Capillas y Moral (2001) fueron: paso 0 = tiempo 0 con un 100% de la fase A, paso 1 = 6 min con 100% fase A, paso 2 = 9 min con 100% fase B y una curva de gradiente Nº 1, paso 3 = 6 min con 100% fase B, paso 4 = 3 min con 100% fase C, paso 5 = 7 min con 100% fase A. El flujo de las fases móviles fue programado a 0,5 mL/min. La temperatura de la columna y de la pre-columna estaba programada a 40° C. La temperatura del coil de reacción fue de 45° C. El flujo del reactivo de post-columna (OPA) fue de 0,3 mL/min. La detección se realizó utilizando un fluorimetro LC 240 (Perkin Elmer, Life and Analytical Sciences, USA) con una longitud de onda de excitación y emisión de 330 nm y 465 nm, respectivamente. El sistema estaba controlado mediante un integrador de datos PE Nelson (Perkin Elmer, Life and Analytical Sciences, USA). La adquisición de datos se realizó con el programa TotalChrom (Perkin Elmer Life and Analytical Sciences, USA).

La identificación y cuantificación de las aminas biógenas se hizo en base a los tiempos de retención por comparación y extrapolación a una curva de calibración realizada mediante diferentes soluciones estándar.

III.2. REFORMULACIÓN DE PRODUCTOS CÁRNICOS

III.2.1. Materias primas

III.2.1.1. Ingredientes cárnicos

Para la preparación de los diferentes productos cárnicos (chorizo y merguez) se empleó carne magra y tocino de cerdo en el caso del chorizo **(capítulo IV.2.1, capítulo IV.2.2, capítulo IV.2.3 y capítulo IV.2.4)** y carne magra y grasa de vacuno para el merguez **(capítulo IV.3.1 y capítulo IV.3.2)**, que se adquirieron en un mercado local de Madrid. Manualmente se eliminó la grasa superficial de la carne magra, y tanto la carne como el tocino se sometieron a un picado de 0,45 cm en una picadora (Mainca, Granollers, España). Posteriormente se colocaron en bolsas de 500-1000 g, se envasaron (Cryovac BB3050) a vacío y se conservaron en congelación (-20 ± 2 ºC) hasta su uso.

III.2.1.2. Ingredientes no cárnicos

III.2.1.2.1. Aceites

Se utilizaron tres tipos de aceite como fuente de grasa no cárnica. Estos fueron: aceite de oliva virgen extra (Carbonell, SOS Cuétara SA, Madrid, España), aceite de linaza (Natursoy SL, Casterçol, España) y aceite de pescado (Omevital 18/12 TG Gold, Cognis GmbH, Illertissen, Alemania). Para la reformulación del chorizo **(capítulo IV.2.3 y capítulo IV.2.4)**, se preparó una mezcla de estos tres tipos de aceite (44,39%, 37,87% y 17,74% de los aceites de oliva, lino y pescado, respectivamente) para obtener un contenido mínimo de 160 mg de EPA/g y 115 mg DHA/g. Para la reformulación del merguez **(capítulo IV.3.1 y capítulo IV.3.2)** se usó solamente el aceite de oliva (Carbonell, SOS Cuétara SA, Madrid, España).

III.2.1.2.2. Konjac

Para la elaboración de los geles de konjac se utilizó harina de konjac (83% de glucomanano, 120 mesh, Trades SA, Barcelona, España), i-carragenato (Secolata IP Hispanagar, Burgos, España), almidón de maíz pre-gelificado (Amigel, Julio Criado Gómez SA, Alcorcón, España) e hidróxido cálcico (Panreac Química SA, Barcelona, España). La preparación del gel se realizó según se indica a continuación (Figura III.3): la harina de konjac fue homogeneizada (Stephan Universal Machine UM5, Stephan Machinery GMBH & Co., Hameln, Alemania) con agua durante 5 minutos; a

continuación se añadió i-carragenato y almidón de maíz pre-gelificado (Amigel, Julio Criado, S.L. Madrid, España) previamente disuelto en agua y homogeneizado, procediendo a su homogenización nuevamente durante 3 min. La mezcla se enfrió hasta los 10 ºC y se añadió una solución de hidróxido cálcico (1%) agitando vigorosamente (a temperatura ambiente) durante 3 min. La mezcla se colocó en un contenedor adecuado y se dejó gelificar durante 24 horas a 2 ºC para su uso posterior.

La elaboración de los otros dos tipos de matrices de konjac empleadas [conteniendo la mezcla de aceites **(capítulo IV.2.3 y capítulo IV.2.4)** y el aceite de oliva **(capítulo IV.3.1 y capítulo IV.3.2)**] se llevó a cabo incorporando el aceite en la matriz del gel de konjac antes de enfriar la mezcla (hasta los 10 ºC), agitando al mismo tiempo durante 3 min. (Figura III.3).

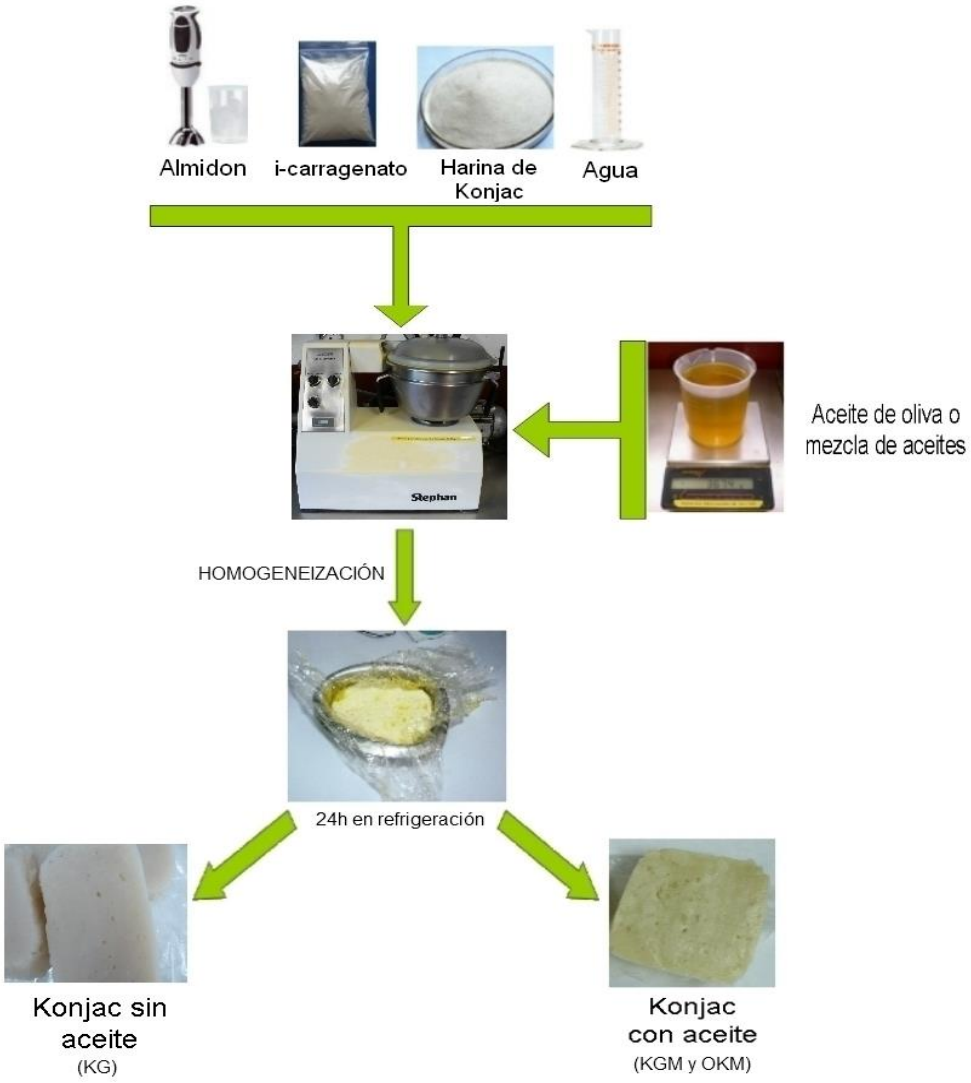

Figura III.3. Proceso de elaboración del gel de konjac (KG) y de la matriz de konjac con aceite (KGM-combinación de aceites y OKM-aceite de oliva)

III.2.1.2.3. Otros ingredientes

En la formulación del chorizo **(capítulo IV.2)** también se emplearon tripolifosfato de sodio (STP) (Manuel Riesgo, SA Madrid, España), nitrito de sodio (Sigma-Aldrich Company Ltd, GmbH), y dos preparaciones comerciales de sales de curado (choravi y curavi) (ANVISA, Arganda del Rey, España).

En la elaboración del merguez **(capítulo IV.3)** se combinaron varios condimentos y especias como son: cilantro (Naturel, Conditionnement de produits agricoles, El Sahlin, Túnez), hinojo (Kamy SA, Nabeul, Túnez), pimentón y pimienta (José Mª Fuster Hernández SA, Murcia, España), menta (DUCROS, Mac Cormick SA, España) y una preparación comercial de Harissa (Ferrero, TUCAL SA, Manouba, Túnez). Dicho preparado de harissa incluye pimentón picante, ajo, cilantro, comino y sal.

Se uso además, cloruro sódico (Panreac Química, SA Barcelona, España) para la elaboración de ambos productos, así como cloruro de potasio, calcio y magnesio (Manuel Riesgo, SA Madrid, España) para la preparación de merguez **(capítulo IV.3.2)**.

III.2.2. Elaboración de los productos

III.2.2.1. Chorizo

Los chorizos fueron diseñados y reformulados para reducir el contenido de grasa y/o mejorar el perfil de ácidos grasos (Figura III.1) con el fin de obtener diferentes niveles de grasa, si bien utilizando la misma cantidad de carne magra, y por lo tanto de proteínas musculares.

En un primer estudio **(capítulo IV.2.1 y capítulo IV.2.2)** se ensayaron 3 formulaciones con distintos niveles de sustitución de grasa animal del 0, 50 y 80% por la misma proporción de gel de konjac (KG) en los lotes NF, RF y LF, respectivamente. En base a los resultados de este primer estudio se abordó un segundo experimento **(capítulo IV.2.3 y capítulo IV.2.4)** en el que se pretendía simultáneamente reducir los niveles de grasa y mejorar el perfil de ácidos grasos de los productos. Para este segundo estudio se empleó como sustituto de la grasa animal el gel de konjac (KG) y una matriz de konjac con la mezcla de aceites (KGM). Se ensayaron 4 formulaciones con distintos niveles de sustitución de grasa animal del 0% y 75% con KG (lotes NF y LFKG, respectivamente), del 90% con KGM (que contenía un 10% de mezcla de aceites) (lote

LFK10) y una sustitución del 100% con KGM (que contenía 20% de mezcla de aceites) (lote LFK20).

Todas las muestras elaboradas en los dos estudios contenían también 5,5%, 0,18% y 1,85% de "choravi", "curavi" y NaCl, respectivamente.

La elaboración de los chorizos se describe en la figura III.4. La carne y el tocino de cerdo fueron descongelados antes de su uso (18 h a 2 ± 2 ºC), picados (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italia) junto con el konjac (0,45 cm) y mezclados manualmente. Esta mezcla se homogeneizó durante 2 min en una amasadora/mezcladora (MAINCA, Granollers, Barcelona, España). Después se añadieron los aditivos (choravi, curavi, NaCl, STP y nitrato de sodio) y se homogeneizaron durante 3 min. En todos los casos la temperatura final fue menor de 11 °C. La mezcla obtenida fue embutida en tripas artificiales de colágeno (Fibran S. A. San Joan de los Abadesses, Girona, España) de 4 cm de diámetro en una embutidora manual (MAINCA, Granollers, Barcelona, España). Los embutidos, de tamaño estándar (22/23 cm) se colocaron en una cámara de maduración (modelo KBF 240 Tuttlingen, Alemania) programada para operar bajo las siguientes condiciones: 48 h a 23 °C y 90% de humedad relativa (RH), seguido de 13 °C, 70 a 80% RH, hasta el final del experimento. El final del proceso de maduración se fijó en 17 días para los chorizos elaborados en el primer estudio **(capítulo IV.2.1 y capítulo IV.2.2)**, mientras para el segundo **(capítulo IV.2.3 y capítulo IV.2.4)**, el proceso de maduración se finalizó cuando la pérdida de peso (en relación con el peso inicial) de los embutidos alcanzó 37-42%. Cuando el experimento lo requería, los chorizos fueron envasados en bolsas de plástico (BB3050 Cryovac ® España) (permeables al O_2) y almacenadas en refrigeración (2 ± 2 °C) durante dos meses para su estudio.

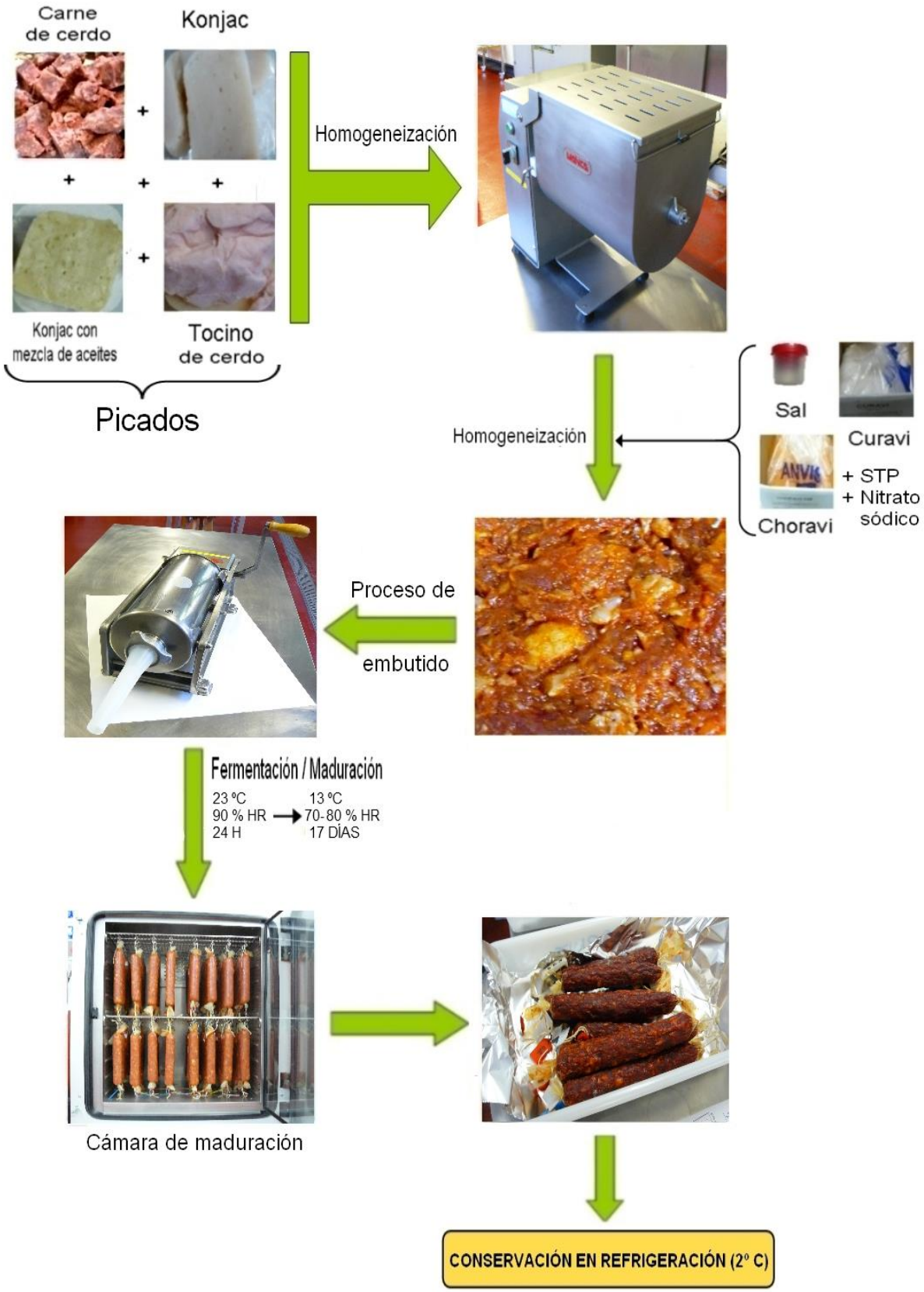

Figura III.4. Procedimiento empleado en la elaboración del chorizo

III.2.2.2. Merguez

Las salchichas frescas tipo merguez fueron diseñadas y formuladas para mejorar el contenido lipídico y reducir el nivel de sodio. Todos los productos contenian cantidades similares de carne magra de vacuno. La reducción del contenido de grasa se hizo sustituyendo la grasa animal con la misma proporción de dos análogos de grasas: gel de konjac (KG) y una mezcla de konjac con aceite de oliva (OKM). En un primer estudio **(capítulo IV.3.1)**, se elaboraron 5 formulaciones, un lote control (C) con contenido de grasa normal (29%), dos lotes sustituyendo el 75% y 100% de la grasa de vacuno con KG (lotes 75/KG y 100/KG, respectivamente) y otros dos lotes con 75% y 100% de sustitución con OKM (lotes 75/OKM y 100/OKM, respectivamente).

En base a los resultados del primer estudio, se ensayó una estrategia de reducción de sodio **(capítulo IV.3.2)** basada en reemplazar el 50% del cloruro sódico añadido en la formulación por una mezcla de sales (MS) que contenía 50% de KCl, 28,58% de $CaCl_2$ y 21,42% de $MgCl_2$. Las formulaciones seleccionadas fueron las que mejores resultados han presentado en el primer ensayo desde un punto de vista tecnológico, sensorial y de estabilidad (75/OKM y 100/KG). Asi se elaboraron 6 productos, 3 con contenido normal de sal que son el control (CNS) y dos lotes, uno similar al 75/OKM (RFNS) y otro al 100/KG (LFNS), y 3 formulaciones de estos 3 lotes con la sustitución del 50% del contenido en sal por la mezcla de sales que fueron CRS, RFRS y LFRS, respectivamente. A fin de aumentar la vida útil de estos productos, se adicionó un conservante, metabisulfito de sódico, en los niveles marcados por la legislación (0,045%) a todos los lotes elaborados de este segundo experimento.

El proceso de elaboración de las salchichas tipo merguez fue como se describe en la figura III.5. La carne y el tocino de vacuno fueron descongelados antes de su uso (18 h a 2 ± 2 ºC), y junto con el konjac fueron mezclados manualmente, picados a 0,45 cm de diámetro (Vam.Dall. SRL. Modelo FTSIII, Treviglio, Italia) y llevados a la amasadora/mezcladora (MAINCA, Granollers, Barcelona, España). A continuación se añadieron el agua y los aditivos, que fueron homogeneizados durante 3 minutos. La temperatura final de la mezcla fue de aproximadamente 6 ºC. La masa fue inmediatamente embutida, utilizando una embutidora manual (MAINCA, Granollers, Barcelona, España), en tripas naturales de cordero de 22 mm de diámetro (tipo C-20/22 Julio Criado Gómez S.A. España) divididas en porciones de un tamaño estándar de 10 ± 2 cm y colgadas en una cámara frigorífica a 2 ºC durante la noche. Al día siguiente, las salchichas fueron colocadas en bandejas de poliespan de EPS (tipo 89 blanco SPT -

Linpac Packaging Pravia, S.A. N R.G.S. España), cubiertas con un film permeable al oxígeno (LINPAC Plastics, Pontivy, Francia) y mantenidas en refrigeración (2 ºC) para su posterior estudio.

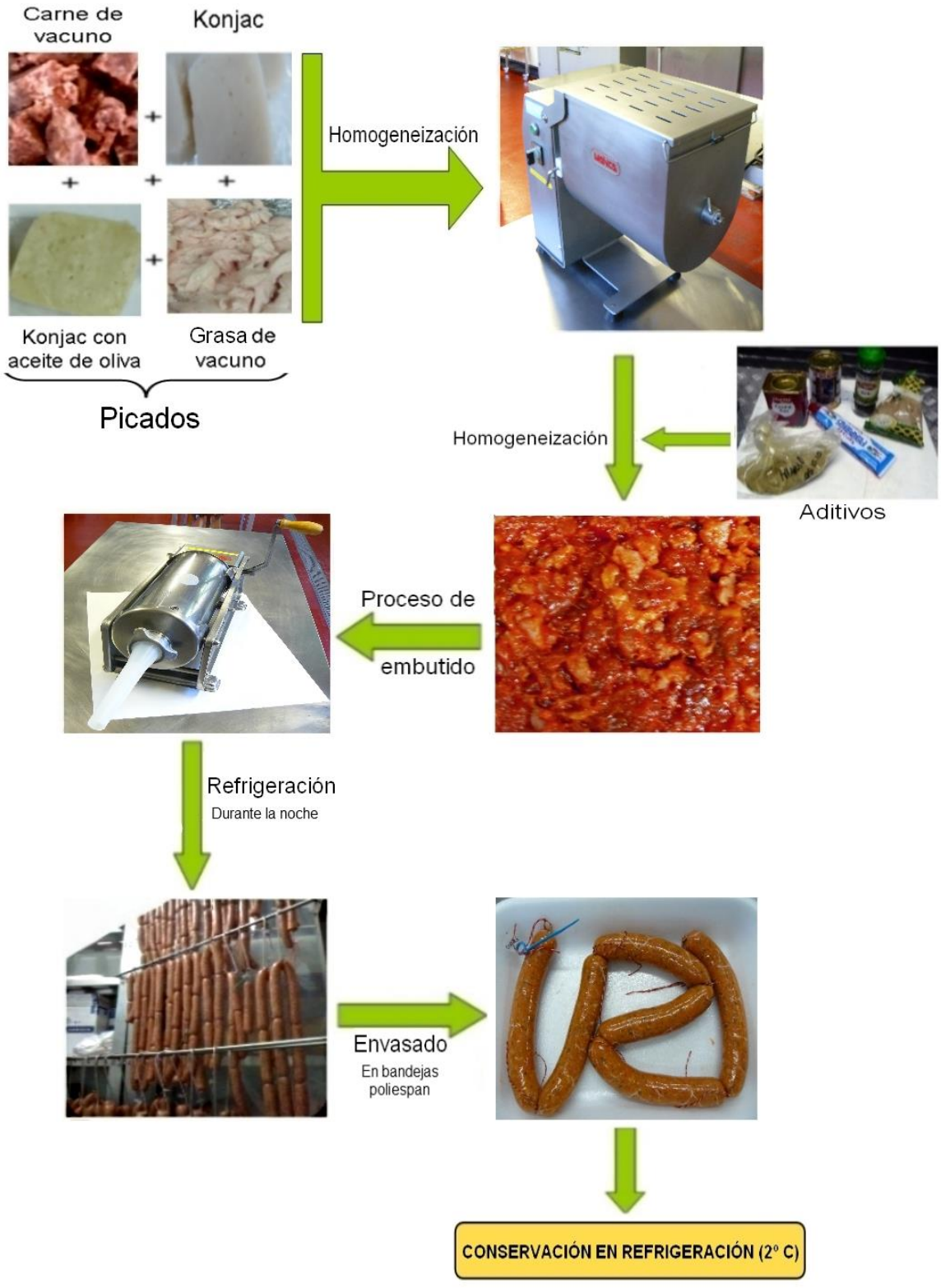

Figura III.5. Proceso de elaboración del merguez

III.2.3. Caracterización de los productos

La viabilidad tecnológica, sensorial y microbiológica de los productos se evaluó analizando distintas propiedades de los mismos. En tal sentido, se realizó su caracterización así como se evaluó su estabilidad a lo largo del procesado y durante la conservación en refrigeración. Igualmente, se llevó a cabo un análisis sensorial para valorar diversos atributos organolépticos.

III.2.3.1. Composición

III.2.3.1.1. Componentes mayoritarios

La determinación de humedad y cenizas (%) se realizó por triplicado según la metodología descrita por la AOAC (2005). La cantidad de proteína (%) se midió por cuadruplicado en un analizador automático de nitrógeno (LECO FP-2000, Leco Corporation, St Joseph, MI, EEUU). El contenido en grasa (%) se determinó por triplicado siguiendo el método descrito por Bligh y Dyer (1959). El contenido en carbohidratos se estimó teniendo en cuenta la composición y formulación de ingredientes de los productos estudiados **(capítulo IV.2.1, capítulo IV.2.2, capítulo IV.2.3, capítulo IV.3.1 y capítulo IV.3.2)**.

III.2.3.1.2. Contenido calórico

El contenido calórico fue estimado tanto en chorizo como en merguez utilizando los factores de conversión de 9,1 kcal/g para la grasa y 4,1 kcal/g para la proteína e hidratos de carbono, respectivamente **(capítulo IV.2.1, capítulo IV.2.3, capítulo IV.3.1 y capítulo IV.3.2)**.

III.2.3.1.3. Perfil de ácidos grasos

La determinación de los ácidos grasos **(capítulo IV.2.3)** se realizó siguiendo el método descrito por Delgado-Pando et al. (2010b). La preparación de los ésteres metílicos se realizó con trifluoruro de boro/metanol (Panreac Química, SA Barcelona, España), siendo analizados en un cromatógrafo de gases Shimadzu (Modelo GC-2014, Kyoto, Japón) equipado con una columna capilar SP-2330 (60 m x 0,25 mm x 0,2 µm) (Supelco, Inc, Bellefonte, EEUU) y un detector de ionización de llama. Las temperaturas del inyector y detector fueron 250 ºC y 260 ºC, respectivamente. La temperatura del horno fue de 140 ºC durante 5 minutos, elevándose hasta los 240 ºC a 4 ºC/min y permaneciendo constante durante 20 minutos. Los ácidos grasos fueron

identificados por comparación con una mezcla estándar de ésteres metílicos (Supelco, Alltech Associated, Inc. Deerfield, IL, EEUU). El análisis se hizo por sextuplicado.

III.2.3.1.4. Minerales

La metodología usada para la determinación de minerales (Ca, K, Na, Mg y Fe) fue la indicada por Serrano et al. (2005) **(capítulo IV.3)**. Las concentraciones de los minerales (mg/100g) se obtuvieron mediante un espectrofotómetro de absorción atómica (Perkin–Elmer, modelo 5100, Norwalk, Connecticut, EE.UU). Se realizaron curvas de calibración para cada elemento. El análisis se hizo por triplicado.

III.2.3.2. Propiedades físico-químicas

III.2.3.2.1. pH

Se determinó el pH por triplicado homogeneizando 10 g de muestra con 100 mL de agua destilada y utilizando para su medida un pH-metro (radiómetro PHM 93, Copenhague, Dinamarca) a temperatura ambiente **(capítulo IV.1.1, capítulo IV.2.4, capítulo IV.3.1 y capítulo IV.3.2).**

III.2.3.2.2. Pérdidas de peso

La determinación de las pérdidas de peso **(capítulo IV.2.1, capítulo IV.2.3, capítulo IV.2.4, capítulo IV.3.1 y capítulo IV.3.2)** se calculó por diferencia de pesada entre el peso inicial y el peso del producto el día de análisis, dividido por el peso inicial y expresado en porcentaje (Triki et al., 2013). El análisis se hizo por triplicado.

III.2.3.2.3. Pérdidas por cocción

Las pérdidas por cocción se realizaron (solamente en el merguez) para estudiar las propiedades ligantes de agua y grasa. Para ello se toman 32 g de muestra (merguez) **(capítulo IV.3)** que se colocaron en tubos falcón y se calentaron a 70 ºC durante 30 min en un baño maría (GRANT, OLS 200, Grant instruments, Cambridge, Ltd., Inglaterra). Tras el tratamiento se determinaron las pérdidas de fluido experimentadas para evaluar los siguientes parámetros:

- Perdidas totales (CL): Peso del fluido liberado tras el calentamiento de la muestra, expresándose como porcentaje con respecto al peso inicial de la muestra.

- Perdidas de agua (WL): Determinado tras la eliminación del agua existente en CL a 105 ºC durante 16 h, expresándose también como porcentaje con respecto al peso inicial de la muestra.
- Perdidas de grasa (%): Calculado como la diferencia entre CL y WL.

III.2.3.2.4. Medida objetiva del color

La evaluación del color se determinó sobre la superficie del corte transversal de los productos **(capítulo IV.2.1, capítulo IV.3.1 y capítulo IV.3.2)**. La estimación objetiva del color se realizó por el método de reflectancia utilizando el sistema de coordenadas CIELab (CIE, 1978; CIE, 1995; Young y Whittle, 1985) mediante un Chroma Meter CR-400 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). Se determinaron los parámetros L*, a* y b*, donde L* representa la luminosidad (0 es negro y 100 es blanco), a* la tendencia al rojo (-60 es verde y +60 rojo) y b* la tendencia al amarillo (-60 es azul y +60 amarillo). Se hicieron quince determinaciones por cada muestra.

III.2.3.2.5. Determinación instrumental de la textura

Para la evaluación de la textura se empleó un texturómetro TA.XT2i (Stable Microsystems Ltd., Surrey, Inglaterra). En el caso de embutidos crudos curados (chorizo), se llevó a cabo el análisis del perfil de textura (TPA). Mientras que en los productos tipo merguez se utilizó un ensayo de compresión/extrusión. El motivo para la elección de uno u otro radicó en la propia naturaleza de los productos. Pese a que el ensayo del perfil de textura proporciona datos más completos, el ensayo de compresión/extrusión se consideró el más adecuado para el merguez debido a su estructura menos sólida.

III.2.3.2.5.1. Análisis del Perfil de Textura (Texture Profile Analysis [TPA])

La evaluación de las características texturales del chorizo **(capítulo IV.2.3 y capítulo IV.2.4)** se realizó mediante TPA de acuerdo al procedimiento descrito por Bourne (1978). Se prepararon porciones de 20 mm de diámetro y 20 mm de altura, que posteriormente se sometieron a doble compresión hasta un 50% de su altura original. Se utilizó una célula de carga de 30 kg a una velocidad del cabezal de 1 mm/s para obtener las curvas de fuerza-tiempo de deformación. Las medidas se realizaron a temperatura

ambiente (22 ºC). A partir de las curvas fuerza-tiempo, se calcularon los siguientes parámetros:

- Dureza (N): definida como la altura máxima obtenida en el primer ciclo de compresión. Este parámetro sirve para evaluar la fuerza máxima necesaria para producir una cierta deformación.
- Cohesividad (adimensional): calculada como la relación entre el área positiva de la primera y segunda compresión.
- Elasticidad (mm): corresponde a la altura recuperada por la muestra tras la primera compresión.
- Masticabilidad (N x mm): calculada como el producto de dureza, elasticidad y cohesividad.

El análisis se hizo por sextuplicado.

III.2.3.2.5.2. Ensayo de compresión/extrusión

Para las muestras de merguez **(capítulo IV.3.2)**, se utilizó el ensayo de compresión/extrusión mediante cizalla utilizando la célula miniatura Kramer/Ottawa y una placa plana de compresión. Las medidas se realizaron, una vez eliminada la piel de las muestras, en porciones de 2,5 cm de largo. Cada una de estas porciones se pesó previamente. Se utilizó una célula de carga de 5 kg. La fuerza de deformación ejercida fue del 50% a una velocidad de cabezal de 2 mm/s. Los resultados se expresaron como el valor de fuerza máxima (fuerza de extrusión, N) respecto al peso (g). La fuerza máxima proporciona información de la consistencia de la muestra. Las determinaciones se realizaron seis veces por muestra a temperatura ambiente (22 ºC).

III.2.3.2.6. Oxidación lipídica

La oxidación lipídica se midió por triplicado **(capítulo IV.2.4, capítulo IV.3.1 y capítulo IV.3.2)** a través de la determinación de las sustancias reactivas con el ácido 2-tiobarbitúrico (TBARs) siguiendo el método de Vyncke (1970) modificado por López-López et al. (2010b). Se realizó una extracción ácida con una solución de ácido tricloroacético (Panreac Química S.A., Barcelona, España) y posteriormente se añadió ácido 2-tiobarbitúrico 20 mM (Merck KGaA, Dannstadt, Alemania). La reacción se llevó a cabo en completa oscuridad durante 20 h a 20 ± 2 ºC. La coloración característica fue medida a 532 nm en un espectrofotómetro (Lambda 15UV/VIS spectrophotometer, Perkin-Elmer, EEUU). Los resultados se calcularon en una curva de

calibración realizada con 1,1,3,3-tetraetoxipropano (Sigma Chemical Co., St. Louis, MO, USA), expresando los resultados en mg de malonaldehído por kg de muestra.

III.2.3.2.7. Actividad de agua (a_w)

La actividad de agua (a_w) se midió (solamente en chorizo) **(capítulo IV.2.1 y capítulo IV.2.4)** durante el procesado y conservación usando un equipo LabMaster-a_W (modelo 1119977, Novasina AG, Lachen SZ, Suiza). Las determinaciones se realizaron por triplicado a 25 ºC.

III.2.3.2.8. Determinación de nitritos

La determinación del contenido de nitrito residual durante el procesado del chorizo **(capítulo IV2.1)** se determinó mediante Análisis de Inyección de Flujo (FIA) (Ruiz-Capillas & Jiménez-Colmenero, 2008). Brevemente, cada muestra fue extraída por duplicado e inyectada en el equipo FIA usando cloruro de amonio como fase portadora. Esta determinación está basada en la reaccion de los nitritos con sulfanilamida para formar una sal de diazonio que se acopla con el N-(1-Naftil)-etilendiamino-dihidrocloruro (NED) para producir un compuesto azoico que se mide por espectrofotometría a una longitud de onda de 540 nm. Los resultados fueron una media de seis determinaciones.

III.2.3.2.9. Determinación de aminas biógenas

El proceso de determinación de las aminas biógenas es el descrito previamente en el apartado III-1.

III.2.3.3. Análisis microbiológico

Se hicieron recuentos de aerobios viables totales, bacterias ácido lácticas y enterobacterias, por duplicado tanto en chorizo **(capítulo IV.2.2 y capítulo IV.2.4)** como en merguez **(capítulo IV.3.1 y capítulo IV.3.2)**, según la metodología descrita por Ruiz-Capillas et al. (2007a). Se pesaron 10 gramos de cada muestra (dos repeticiones por muestra) en bolsas estériles de plástico con 90 mL de agua de peptona. Las bolsas se colocaron en el equipo estomacher (Stomacher Colworth 400, Seward, Inglaterra) durante 2 minutos. A partir de la solución obtenida se hicieron las diluciones decimales para la siembra posterior en función de los análisis microbiológicos a realizar.

Para el recuento de microorganismos aerobios viables totales se utilizó el medio de cultivo PCA (Plate Count Agar) incubando a 30 ºC durante 72 h. La determinación de bacterias ácido lácticas se llevó a cabo en MRS (Mann Rogosa Sharp Agar) incubando a 30 ºC entre 3 y 5 días, mientras que el recuento de enterobacterias se efectuó sobre el medio de cultivo VRBG (Violet Red Bile Glucose Agar) incubando a 37 ºC durante 24 h.

III.2.3.4. Análisis sensorial

El análisis sensorial, específico para cada producto cárnico, se llevó a cabo para evaluar sus propiedades organolépticas y comparar su grado de aceptación frente a los respectivos controles. La selección de los catadores, se realizó con diversas sesiones de entrenamiento en las que los panelistas se familiarizaron con los productos y los atributos a medir. Finalmente se eligieron 15-20 panelistas para el análisis sensorial. Para cada formulación se evaluaron diferentes parámetros en una escala no estructurada de 0 a 10 sin extremos fijos. Los parámetros considerados en chorizo **(capítulo IV.2.1 y capítulo IV.2.3)** fueron: apariencia, jugosidad, dureza, sabor y aceptabilidad general. Mientras que en el caso del merguez **(capítulo IV.3.1 y capítulo IV.3.2)** se evaluaron la jugosidad, dureza y aceptabilidad general.

III.2.4. Análisis estadístico

El análisis estadístico de los resultados se realizó empleando el SPSS Statistics 13.0, 14.0 y 17.0 (SPSS Inc, Chicago, Estados Unidos).

Para determinar el efecto de la formulación sobre los diferentes parámetros estudiados, se realizó un análisis de la varianza (Analysis of variance, ANOVA) de una vía. Se utilizó el test “post hoc” de la diferencia significativa de Tukey (Honest Significant Difference, HSD) para determinar las diferencias entre grupos. Para establecer el efecto de las diferentes formulaciones y el tiempo de conservación en los parámetros medidos, se utilizó un ANOVA de dos vías (formulación y tiempo) donde se empleó el test HSD de Tukey para determinar las diferencias. También se utilizó el cálculo de la correlación de Pearson para establecer la posible relación entre dos variables. El nivel de significación quedó establecido en $P<0,05$ para todos los análisis.

IV. TRABAJO EXPERIMENTAL

IV.1. OPTIMIZACIÓN DEL MÉTODO DE DETERMINACIÓN DE AMINAS BIÓGENAS EN CARNES Y PRODUCTOS CÁRNICOS

IV.1.1. Optimisation of a chromatographic procedure for determining biogenic amine concentrations in meat and meat products employing a cation-exchange column with a post-column system.

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Analytical Methods

Optimisation of a chromatographic procedure for determining biogenic amine concentrations in meat and meat products employing a cation-exchange column with a post-column system

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ABSTRACT

The aim of this study was to optimise and validate a chromatographic method for determining biogenic amine (BA) in meat and meat products separated by a cation-exchange column with a post-column system, using o-phthalaldehyde as a derivatising reagent. A perfect separation of nine BA (tyramine, histamine, β-phenylethylamine, putrescine, cadaverine, tryptamine, agmatine, spermidine and spermine) was obtained in 22 min. The conditions were: column T^a 40 °C and coil T^a 45 °C, pump flow rate 0.8 mL/min, pH phase A 6.33, B 5.63 and C 13.00. The method was adjusted linearly in a range of 0.10–12 mg/L with a correlation coefficient superior to 0.998. Detection and quantification limits were between 0.03–0.10 mg/L and 0.10–0.20 mg/L, respectively. Precision studies were satisfactory, with RSD less than 2% and meat extracts recovering over 98%. This method showed an appropriate, precise, fast and versatile procedure for determining nine BA simultaneously in different meat product matrices.

1. Introduction

Biogenic amines (BAs) are compounds that are present in a large number of foods, including meat and meat products. These compounds are classified in three categories, according to their chemical structure: aromatic amines (histamine, tyramine, serotonin, β-phenylethylamine, and tryptamine), aliphatic diamines (putrescine and cadaverine), and aliphatic polyamines (agmatine, spermidine, and spermine) (Smith, 1980). Biogenic amines are formed by decarboxylation of free amino acids (FAAs) as a result of amino acid decarboxylase enzyme activity (Miet & Karmas, 1978; Smith, 1980). Biogenic amine concentrations are conditioned by numerous factors such as FAA content and availability, microorganisms capable of producing decarboxylases, the nature of the medium (pH, ion strength, etc.), processing and preservation conditions, etc. (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Ruiz-Capillas & Jiménez-Colmenero, 2004a; Smith, 1980).

Determination of the BA content in foods is of interest not only because of its toxicological implications but also for its utilisation as a food quality index, since some biogenic amines can pose health risks when ingested in high quantities. The consumption of foods with high levels of tyramine and histamine can be toxic (Bardócz, 1995; Shalaby, 1996; Smith, 1981), therefore tryptamine and β-phenylethylamine are also included in the vasoactive amine group that causes a rise in blood pressure (Koehler & Eithenmiller, 1978). Other biogenic amines such as putrescine and cadaverine, although not posing a direct risk, can also have negative effects on health since their actions stimulate the toxicological effect of tyramine and histamine (Smith, 1980). Although, under normal conditions the human body can quickly absorb the amines in food, both high ingestion of amines or damage to the amine-oxidase enzyme can lead to their accumulation in the body and cause serious toxicological problems (Halász et al., 1994).

As changes in some biogenic amine concentrations occur during muscle food processing and storage, biogenic amine determination has been employed on a regular basis as a meat and fish quality index (Halász et al., 1994; Ruiz-Capillas & Jiménez-Colmenero, 2004a; Ruiz-Capillas & Moral, 2001). Its usefulness in this role will depend on factors associated with food composition, processing and storage conditions.

Several procedures have been developed and improved for appropriate, accurate and fast analytical detection and determination of biogenic amines in different foods (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou, 1996; Hurst, 1990; Ruiz-Capillas & Jiménez-Colmenero, 2009; Önal, 2007). Most of these methods are based on chromatographic (HPLC) determinations using pre- and post-columns with reverse phase or ion exchange columns. Different derivatising reagents such as ninhydrin, dansyl chloride and ortho-phthalaldehyde (OPA) have been used, depending on the type of column (Eerola, Hinkkanen, Lindfors, & Hirvi, 1993; Hernández-Jover et al., 1996; Hurst, 1990; Ruiz-Capillas

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& Jiménez-Colmenero, 2009; Smělá, Pechová, Komprda, Klejdus, & Kubáň, 2003; Önal, 2007). The derivatising reagents also condition the detection system, and various detection systems were used such as UV/Vis, diode array, fluorescent etc. The latter is the most widely used in the literature Hurst, 1990; Izquierdo-Pulido, Vidal-Carou, & Mariné-Font, 1993; Önal, 2007; Ruiz-Capillas & Jiménez-Colmenero, 2009).

Most of these procedures provide low sensitivity and detection, only a limited number of amines are simultaneously separated, and the equipment employed is expensive etc. Even though BA determination in food is not a simple procedure, the analytical difficulties are further complicated in meat products owing to factors such as the complexity of the meat matrices (protein-rich food, often with high-fat content and the presence of numerous non-meat ingredients of animal or plant origin) as well as the different processing procedures they have undergone. Apart from the foregoing, it is necessary to take into account the diverse chemical structures of BA and the wide range of concentrations of each BA in these products, especially β-phenylethylamine and tryptamine. These two amines are very important for toxicological reasons since their high concentrations, principally β-phenylethylamine, in meat and meat products are produced by microorganisms, mainly lactic acid bacteria (de las Rivas et al., 2008; Ruiz-Capillas, Jiménez-Colmenero, Carrascosa, & Muñoz, 2007).

Among the different methods tested for determining biogenic amines in muscle foods is the procedure developed by Ruiz-Capillas and Moral (2001). This method is based on selective, sensitive and efficient separation on a cation-exchange column, a robust procedure because it does not suffer hydrolytic degradation (Tracy, Pickering, & Verhulst, 1995). This technique also uses a derivatisation post-column system with OPA to get a colorimetric reaction detected by fluorescence. The use of OPA as a derivatising agent increases the sensitivity of most amines, which is not the case with other detection procedures (Eerola et al., 1993; Izquierdo-Pulido et al., 1993; Tracy et al., 1995). This type of automated post-column derivatisation also prevents possible interference introduced by performing pre-column derivatisations and economises on time needed for the preparation of samples.

The object of this work was to establish experimental conditions which would optimise and validate a chromatographic method for the determination of biogenic amines in meat and meat products. The intention was to determine simultaneously the concentrations of nine biogenic amines (tyramine, β-phenylethylamine, histamine, putrescine, cadaverine, tryptamine, agmatine, spermidine and spermine), together with two additional ones (β-phenylethylamine and tryptamine) based on the original method (Ruiz-Capillas & Moral, 2001). An additional objective was to improve the sensitivity, selectiveness and efficiency of BA separation with respect to different complex matrices such as fresh meat, Spanish fermented sausage (“chorizo”) and frankfurter. These products were chosen as they represent the various types of meat products which show different levels of amines associated with different processing and storage conditions.

2. Materials and methods

2.1. Reagents

The trichloroacetic acid (TCA) was supplied by Panreac (Barcelona, Spain) and the ultra pure water was obtained from Milli-Q system (Millipore, France). Methanol and 2-propanol for high liquid chromatography, potassium phosphate dibasic, potassium hydroxide, potassium chloride, acetic acid and Brij 35 solution (30% w/v) were from Sigma–Aldrich (Spain). The following materials were purchased from Pickering Laboratories (CA, USA): o-phthalaldehyde (OPA, Ref. O120), Thiofluor Chromatographic Grade (*N*,*N*-dimethyl-2mercaptoethylamine-hydrochloride), OPA diluent (Ref. OD104: 3% potassium hydroxide, 3% boric acid, 94% water, pH 10.40), and for the mobile phases, potassium phosphate phase A buffer (Ref. K600: 11% 2-propanol, 0.9% potassium phosphate dibasic, 0.3% acetic acid, 87.8% water, pH 6.00), phase B (Ref. K563: 5% potassium chloride, 4% 2-propanol, 0.9% potassium phosphate dibasic, 0.3% acetic acid, 89.8% water, pH 5.63) and the potassium regenerating column, phase C (Ref. K130: 0.7% potassium chloride, 4% 2-propanol, 0.5% potassium hydroxide, 94.8% water, pH 13.00). The post-column derivate reagent OPA was prepared with 975 mL of OPA solution (OD 104) and 0.100 mg of OPA dissolved in 10 mL methanol, 2 g Thiofluor and 3 mL of Brij 35 solution.

2.2. Standard solutions

The standard solutions of biogenic amines which were the subject of this study, namely, tyramine hydrochloride (Tyr), histamine dihydrochloride (His), 2-phenylethylamine hydrochloride (Pea), putrescine dihydrochloride (Put), cadaverine dihydrochloride (Cad), tryptamine-crystalline (Try), agmatine sulphate salt (Agm), spermidine trihydrochloride (Spd) and spermine tetrahydrochloride (Spm) were purchased from Sigma–Aldrich (Spain).

A stock solution of 1000 mg of each BA/L was prepared, as a free base with TCA 7.5%, to use in calibration studies. From this, a 100 mg/L intermediate solution including all biogenic amines was also prepared with TCA 7.5% from the stock solution. Then, from this intermediate solution the appropriate standard solutions from 0.05 to 12 mg/L of mixed BAs were prepared with TCA 7.5% to prepare the calibration standards. The standard solutions were also filtered through a 0.22 μm Nylon Syringe filter (Teknokroma, Barcelona, Spain) into 2 mL amber vials with screw caps (PTFE/silicone) (Perkin Elmer Life and Analytical Sciences, USA) and then placed in the auto-sampler to be injected into a high-performance liquid chromatograph (HPLC) as described below (Perkin Elmer Life and Analytical Sciences, USA).

2.3. Meat products and sample preparation

Fresh pork (*Longuissimus dorsi*), Spanish fermented sausages (chorizo) and frankfurter were selected because of their different characteristics and purchased in a local market. They were chosen for being representative of the different meat products widely accepted by Spanish consumers, though with different types of matrices (structural characteristics and composition), processing and marketing conditions (fresh, fermented and cooked), and containing a wide range of BA levels. This experimental design would make it possible to test the effectiveness of the method over a wide range of real conditions.

The BA extraction process was carried out by blending 13–16 g of each sample with 30 mL of 7.5% trichloroacetic acid in an omnimixer (Omni Internacional, Waterbury, CT, USA) (20,000 rpm, 3 min) and centrifuged at 5000*g* for 15 min at 4 °C in a desktop centrifuge (Sorvall RTB6000B, DuPont, USA). The supernatants were filtered through a Whatman No. 1 filter, passed back through a 0.22 μm Nylon filter (Millipore, Ireland) and then placed in opaque vials in the auto-sampler and stored at 2 °C, until use (within the next 24 h).

2.4. pH determination of mobile phases

The pH of the mobile phases was determined using a pH meter (Radiometer PHM 93, Copenhagen, Denmark).

2.5. Chromatographic analysis of biogenic amines

The chromatographic determination of BAs was done using liquid chromatography consisting of a quaternary pump (series 200, Perkin Elmer, SL Spain), an auto-sampler (series 200, Perkin Elmer Life and Analytical Sciences, USA), a Pickering PCX 3100 post-column system (Pickering Laboratories, CA, USA) containing a cation-exchange column (K^+, 4 mm × 150 mm) with a 10 μm particle diameter and a pre-column (K^+, 3 mm × 20 mm) also with a 10 μm diameter particle (Pickering Laboratories, CA, USA) located in a Pickering PCX 3100 post-column system (Pickering Laboratories, CA, USA). The whole system was degassed and pressurised with helium. The BA were eluted using an elution gradient programmed with potassium eluents K600, K563 and K130 following the method used by Ruiz-Capillas and Moral (2001) with a lineal gradient (without curve) programme except in step 2 where an isocratic gradient curve was applied (curve No. 1) as follows: step 0 time = 0 min with 100% A, step 1 time = 6 min with 100% A, step 2 time = 9 min with 100% B and a gradient curve No. 1, step 3 time = 6 min with 100% B, step 4 time = 3 min with 100% C, step 5 time = 7 min with 100% A. The mobile phase flow was programmed at 0.5 mL/min. The column and pre-column temperatures were programmed at 40 °C. In the reaction chamber, the post-column reagent (OPA) flow was 0.3 mL/min. The temperature of the reaction chamber was kept at 45 °C. Detection was done using an LC 240 fluorescence detector (Perkin Elmer Life and Analytical Sciences, USA) at 330 nm excitation and 465 nm emission. All the chromatographic systems were controlled using a PE Nelson data integrator (Perkin Elmer Life and Analytical Sciences, USA). Data acquisition was carried out using TotalChrom software (Perkin Elmer Life and Analytical Sciences, USA).

Amine identification and quantification was done by comparing and extrapolating retention times with a calibration curve performed with different standard solutions.

2.6. Statistical analysis

Data were presented as the mean, standard deviation (SD) and relative standard deviation (RSD). Linear regressions were performed, with concentration as a dependent variable and time as an independent variable. The software used for these analyses was the statistical package SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA).

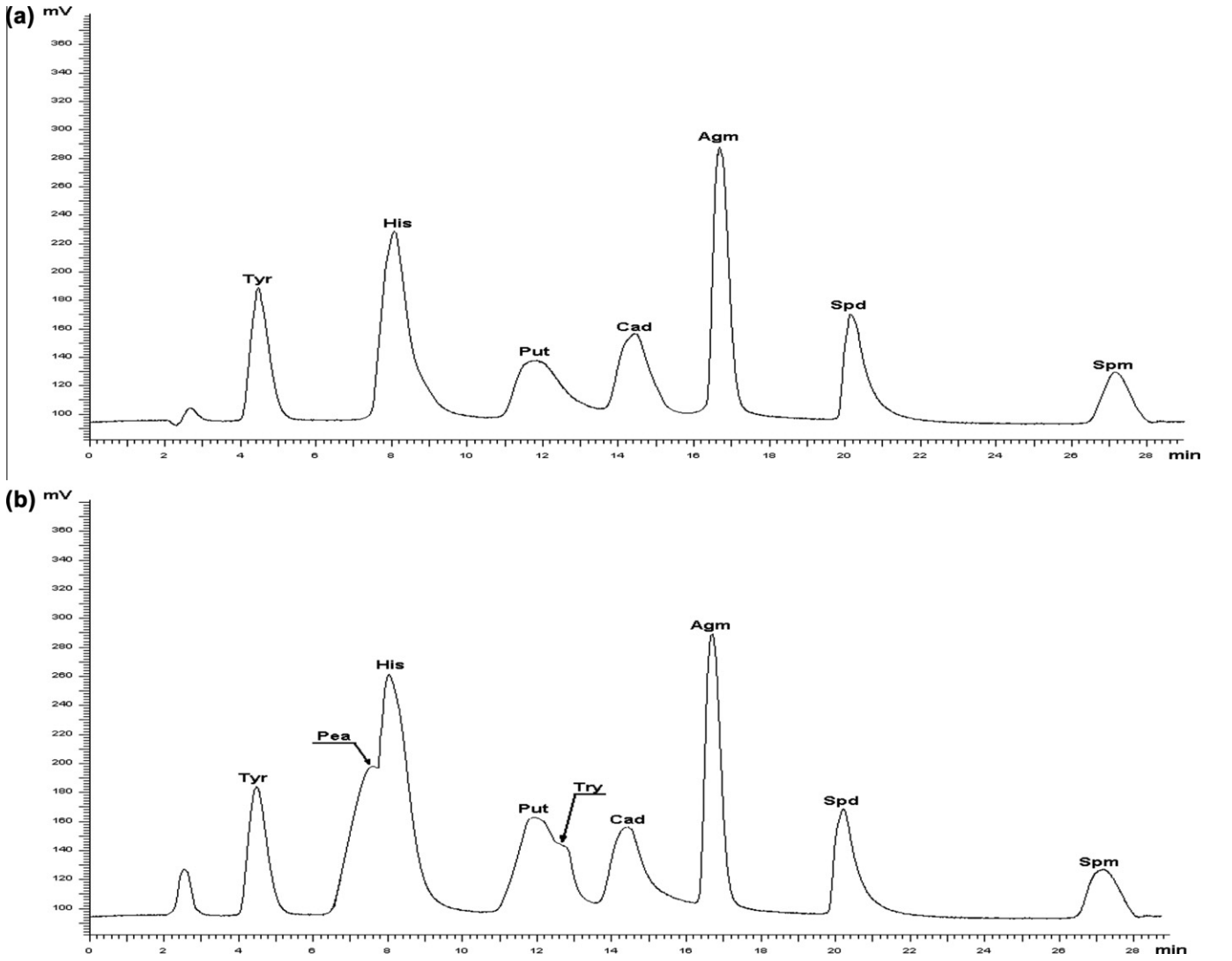

Fig. 1. Chromatograms obtained using the methodology of Ruiz-Capillas and Moral (2001) (a) for standard solution of 4 mg/L of biogenic amines (tyramine, histamine, putrescine, cadaverine, agmatine, spermidine and spermine) and (b) for standard solution of 4 mg/L of biogenic amines plus the two new amines (β-phenylethylamine and tryptamine).

Table 1
Gradient elution program for the chromatographic separation of biogenic amines in meat and meat products.

Step	Time (min)	Flow (mL/min)	Mobile phases			Gradient curve
			A (%)	B (%)	C (%)	
0	0	0.8	100	0	0	0
1	6	0.8	100	0	0	0
2	9	0.8	0	100	0	1
3	6	0.8	0	100	0	0
4	3	0.8	0	0	100	0
5	7	0.8	100	0	0	0

3. Results and discussion

3.1. Method optimisation

A typical chromatogram of a combination of the seven BAs most often determined (tyramine, histamine, putrescine, cadaverine, agmatine, spermidine and spermine) (each one at 4 mg/L) using the original Ruiz-Capillas and Moral (2001) method is shown in Fig. 1. The chromatogram presents an acceptable resolution (Ruiz-Capillas & Moral, 2001). However, when β-phenylethylamine, and tryptamine (also at 4 mg/L) were added to the aforementioned combination of BAs under the same methodological conditions, it was evident from the chromatogram that these two additional amines were not properly resolved (Fig. 1). β-Phenylethylamine coincided with histamine and is unresolved properly, with an elbow at the histamine peak. Tryptamine coincided with putrescine, at the end of the peak, and is also unresolved adequately. In numerous publications (de las Rivas et al., 2008; Hernández-Jover et al., 1996; Roig-Sagués, Ruiz-Capillas, Espinosa, & Hernández, 2009; Smělá et al., 2003) the levels of these two amines, principally β-phenylethylamine, in meat products has been attributed to the action of a variety of microorganisms and strains. *Staphylococcus carnosus* strains analysed in pressurised dry-cured Spanish "chorizo" sausage produced either β-phenylethylamine alone or both β-phenylethylamine and tyramine together (de las Rivas et al., 2008).

In order to avoid such problems and improve the sensitivity, selectiveness and efficiency of BA separation, different methodological changes were made and tested in relation to column temperature, pH of the reagents A and B, programme time, gradient curves and the pump flow rate for the mobile phases in order to reduce analysis time. As the ion exchange column temperature is critical in the separation of amines (Tracy et al., 1995; Vidal-Carou, Lahoz-Portolés, Bover-Cid, & Mariné-Font, 2003), lower (35 °C) and higher (45 °C) temperatures were tested when scheduling the original method (40 °C), keeping the temperature of the reaction coil fixed at 45 °C. By decreasing the column temperature to 35 °C there was a slight improvement in the resolution that was not observed at 45 °C, but this was not considered to be sufficient so the temperature was maintained at 40 °C. Similar column temperatures (42 °C, 45 °C) had already been tested (Latorre-Moratalla et al., 2009; Tracy et al., 1995). Flow rates of 0.6 and 0.8 mL/min which were tested with the aim of shortening analysis time did nothing to improve the separation of the two new amines but a 5-min reduction in elution time was achieved with the flow of 0.8 mL/min. However, a total time of 30 min was needed to regenerate the column in order to obtain a stable balance for subsequent determinations (Table 1). Further test programmes with linear gradients and different gradient curves (−6, −2, −1, −4, 2, 1) were applied, but none of these programmes produced significant improvements in β-phenylethylamine and tyramine resolution. Therefore, curve 1 was the selected gradient in the second step of the pump programme similar to the original (Table 1).

Since, in chromatographic separation in ion exchange columns, the pH of the mobile phases is a key factor in resolving the various peaks (Hernández-Jover et al., 1996; Tracy et al., 1995; Vidal-Carou et al., 2003), the effects produced by these pH variations during the mobile phases were taken into account. The pH changes in phases A and B were compared with their starting pH (6 and 5.63, respectively). A phase pH was increased to 6.4 by 1 N NaOH and B phase pH to 6 by 2-propanol. A phase pH increases produced changes in the order of the targeted amine elution peaks. In the case of the β-phenylethylamine-histamine pair, the histamine peak came first followed later by that of β-phenylethylamine. In the case of tryptamine, the pH increase made its peak coincide with that of cadaverine instead of putrescine. Nevertheless, this pH increase effectively separated histamine and β-phenylethylamine though it failed to separate tryptamine from cadaverine and the chromatogram baseline linearity could not be maintained. In order to avoid possible baseline modification the mobile B phase pH was fixed at

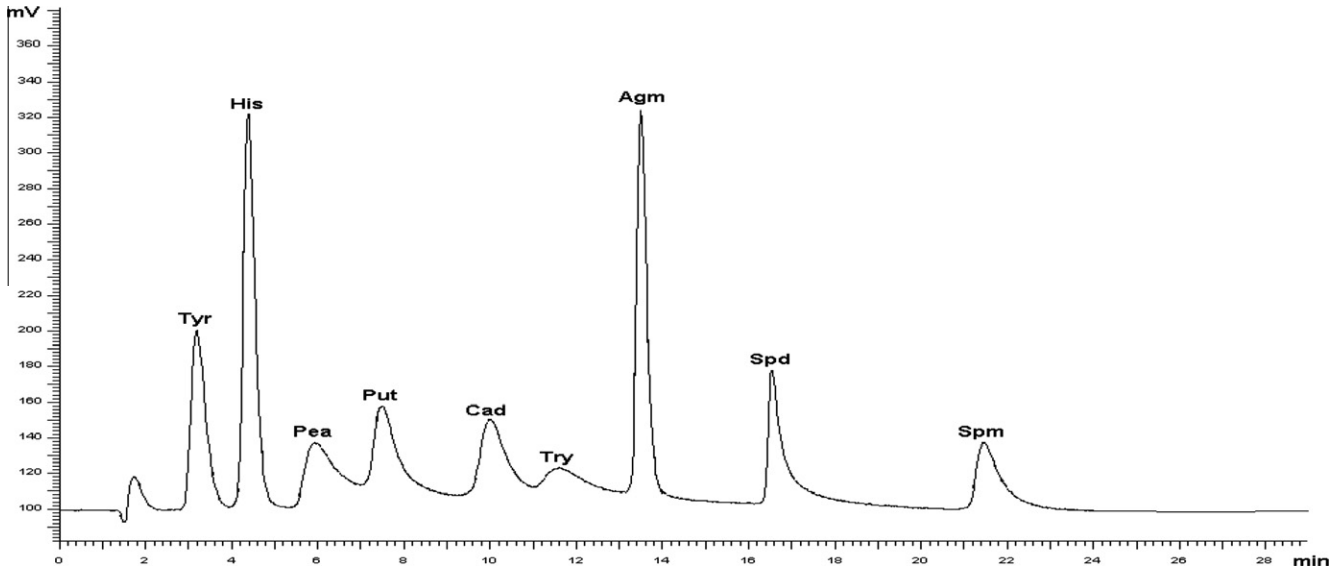

Fig. 2. Chromatogram of a standard solution of 4 mg/L of biogenic amines (tyramine, β-phenylethylamine, histamine, putrescine, cadaverine, tryptamine, agmatine, spermidine and spermine) using the optimised methodology.

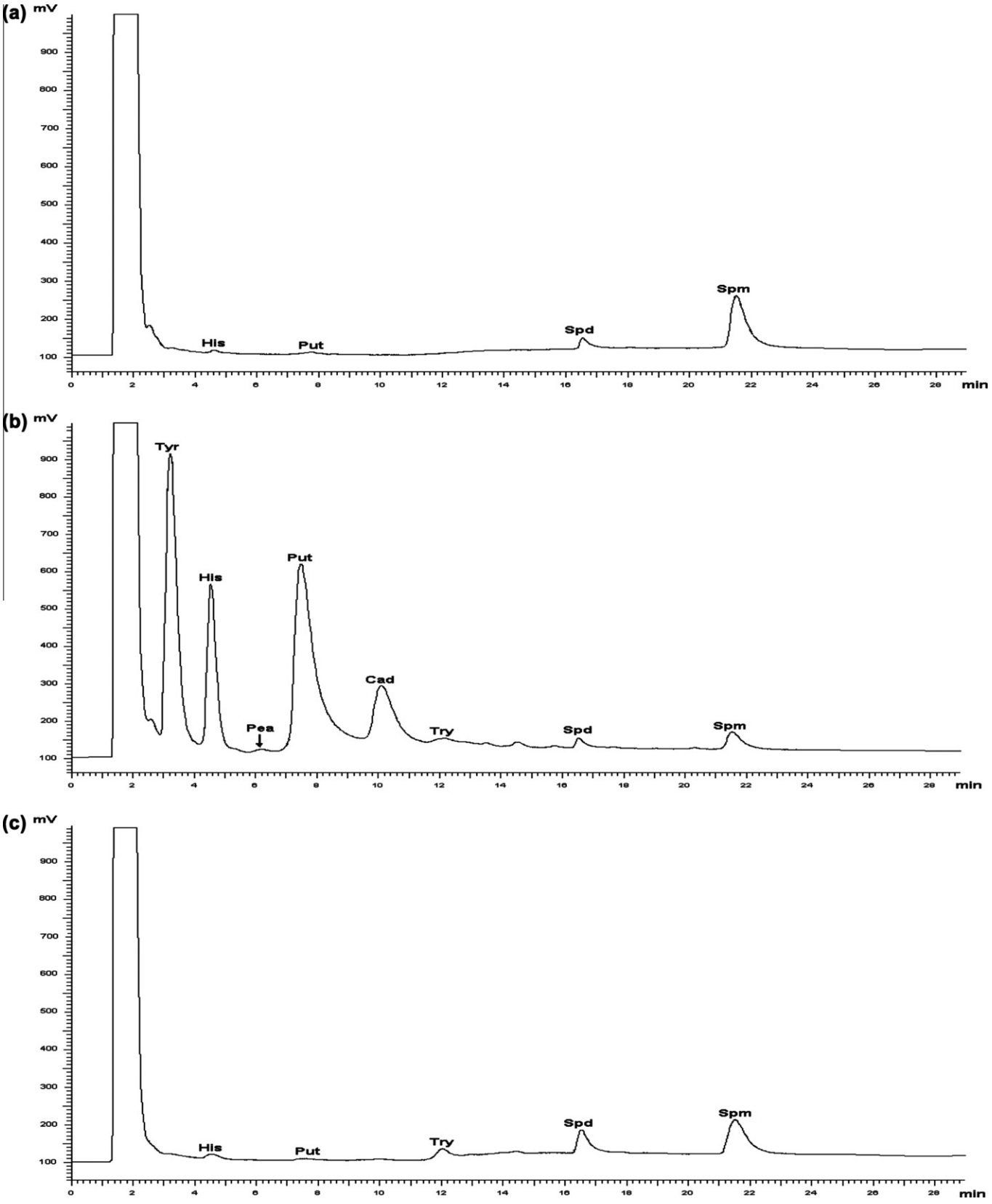

Fig. 3. Chromatograms of biogenic amines (tyramine, β-phenylethylamine, histamine, putrescine, cadaverine, tryptamine, agmatine, spermidine and spermine) in meat products (a: fresh meat, b: "Chorizo" and c: Frankfurter) using the optimised methodology.

5.63 and A phase pH changes were studied between 6.00 and 6.40. After the study it was observed that an A phase pH of 6.33 was the most suitable for perfect amine separation and for maintaining an acceptable baseline. To improve stabilisation, pH adjustments during the mobile phases were carried out with acetic acid instead of NaOH because stability during phase A was short-lived when the

pH was adjusted using NaOH. Other authors have also used acetic acid for pH adjustments in the mobile phases for biogenic amines in cation-exchange column separation (Hernández-Jover et al., 1996; Tapia-Salazar, Smith, & Harris, 2000).

Optimal separation of amines (Fig. 2) was achieved with an A phase pH of 6.33, and by maintaining the original B and C phase pH of 5.63 and 13.00, respectively, Together with the A phase pH of 6.33 the other optimal methodological conditions selected were a column temperature of 40 °C, a coil temperature of 45 °C and a pump flow rate of 0.8 mL/min, applying the elution programme described in Table 1.

An appropriate baseline was maintained, and a perfect separation of the biogenic amines (tyramine, histamine, β-phenylethylamine, putrescine, cadaverine, tryptamine, agmatine, spermidine and spermine) was obtained in 22 min, reducing the BA elution time to 5 min although total programme time had to remain at 31 min to achieve a correct recovery of the column (Fig. 2). The analysis time is similar to that of other authors (Tapia-Salazar et al., 2000) and even much shorter if compared to other studies of between 40 and 120 min (Hernández-Jover et al., 1996; Lavizzari, Veciana-Nogués, Bover-Cid, Mariné-Font, & Vidal-Carou, 2006; Standara, Vesela, & Drdak, 2000; Vidal-Carou et al., 2003).

The chromatographic profile of the meat and meat product samples analysed are shown in Fig. 3, together with a selective, prompt and appropriate resolution of each amine peak without interference.

3.2. Validation of the method

Table 2 shows the equations of the regression lines and correlation coefficients for each amine studied in a range of 0.10–12 mg/L (four replicates were taken), thus facilitating an evaluation of the linearity of the method in the proposed range. The correlation coefficients (R^2) for all the amines were equal to or higher than 0.9990 except for Cad, Try and Spm which were 0.9978, 0.9956 and 0.9985, respectively. These coefficients are similar to those observed by other authors who have studied amine determination techniques and in the case of β-phenylethylamine and tryptamine are even higher depending on the methodology under consideration (Hernández-Jover et al., 1996; Lavizzari et al., 2006; Sánchez & Ruiz-Capillas, 2005; Vidal-Carou et al., 2003).

The sensitivity of the method was estimated from the detection (LOD) and quantification (LOQ) limits. The detection limits, calculated on the regression curve, were done using standard solutions of low concentration from 0.05 to 1 mg/L for all amines. Both the detection and quantification limits were calculated as the sum of the average concentration of the blank plus 3 and 10 times, respectively the standard deviation of the blank obtained with 10 determinations. Detection limits were between 0.03 and 0.10 mg/L, the lowest being observed in the amines that appeared in the first part (10 min) of the chromatogram.

Table 2
Linearity: regression curve and regression coefficient (R) for the determination of different biogenic amines in a standard solution ranging from 0.1 to 12 mg/L.[a]

Biogenic amines	Regression curve	Regression coefficient (R^2)
Tyr	$y = 0.984x + 0.0134$	0.9990
His	$y = 0.983x + 0.0028$	0.9991
Pea	$y = 0.9911x + 0.003$	0.9993
Put	$y = 0.9803x + 0.0218$	0.9991
Cad	$y = 0.9921x - 0.0643$	0.9978
Try	$y = 0.9953x - 0.0347$	0.9956
Agm	$y = 0.988x - 0.0285$	0.9992
Spd	$y = 1.0156x - 0.0468$	0.9992
Spm	$y = 1.0098x - 0.099$	0.9985

[a]The regression curve and the regression coefficient were obtained from an average of four replicates.

Quantification limits were in the range of 0.10 mg/L for tyramine, histamine, β-phenylethylamine and putrescine; in the range of 0.15 mg/L for agmatine, cadaverine, tryptamine and spermidine and 0.2 mg/L for spermine. Detection and quantification limits of this optimised method are lower than those of the original method (Ruiz-Capillas & Moral, 2001), that had detection and determination limits below 0.05–0.08 mg/L and 0.2 mg/L, respectively. The results observed in this study are similar and even lower than those observed by other authors that have employed post-column derivatisation (Hernández-Jover et al., 1996; Sánchez & Ruiz-Capillas, 2005; Tracy et al., 1995) and almost similar to those observed in one ultra-high pressure liquid chromatographic quantification (Latorre-Moratalla et al., 2009), though slightly higher than those observed by other authors (Lavizzari et al., 2006; Tapia-Salazar et al., 2000).

The accuracy of the method and the instruments used was determined by means of repeatability (retention time and BA concentration). Repeatability studies of different standard retention times were assessed using eight consecutive analyses of different standards (0.1–12 mg/L) with the nine BAs employed for carrying out the calibration curves. The standard deviations of the different standard retention times were very low $\leqslant 0.06$ for all amines except tryptamine that was 0.19. Again, the RSDs were between 0.10 and 0.83 for all amines except for tryptamine which was an acceptable 1.66. This low variability in retention times was also observed in the meat, frankfurter and "chorizo" samples studied, whether unspiked or spiked with known amounts of 20 mg/kg of each biogenic amine standards in the extract. These samples presented standard deviations of the retention times that were similar to the standards and an RSD between 0.19 and 0.93 for all amines except tryptamine which was 1.57. This is very important and shows that, with this method, BA identification in the chromatogram can be carried out on the basis of retention time by comparison with the standard solution, without the need to use internal standards for amine quantification. The chromatographic determination of BAs in complex samples, as it is the case with meat and meat products, can cause significant movements in retention times of various amines, due to the front, forcing the use of internal standards for amine identification and quantification (Eerola et al., 1993).

The repeatability of the method with respect to BA concentration was studied by evaluating eight consecutive analyses of the standard solution of 4 mg/L of all biogenic amines and meat samples analysed, whether spiked or unspiked (Table 3). The standard deviation relative to concentrations in the standard solution (4 mg/L) of biogenic amines was less than 1% in all cases. The highest values were for cadaverine and tryptamine with RSDs of 0.63% and 0.89%, respectively. The RSD for spiked samples was always less than 3%. The repeatability results observed using this method registered lower percentages than those of the original method (5%), which were already considered appropriate. They were also lower than values observed in other BA determination studies employing HPLC with post-column derivatisation and OPA (Hernández-Jover et al., 1996; Sánchez & Ruiz-Capillas, 2005; Vidal-Carou et al., 2003).

Recovery studies of the amounts of amines present in the extracts of the samples analysed (fresh meat, "chorizo" and frankfurter) were performed by means of spiking extracts with known amounts of 20 mg/kg of each biogenic amine (Table 3). The mean recovery of these extracts was higher than 98% for each amine, similar to that observed by other authors studying amine determination in meat products and in solution (Hernández-Jover et al., 1996; Latorre-Moratella et al., 2009). The accuracy of the method was considered appropriate for this type of samples.

With regard to BA levels in the meat samples studied, it should be noted that the greatest concentrations were observed in "cho-

Table 3
Repeatability of biogenic amines in the standard solution of 4 mg/L and in the extract of fresh meat, "chorizo" and frankfurter samples.[a]

BA	Patron 4		Fresh meat				"Chorizo"				Frankfurter			
	Media (mg/L)	RSD (%)	Initial content (mg/kg)	Content after addition (mg/kg)	RSD (%)	Recovery (%)	Initial content (mg/kg)	Content after addition (mg/kg)	RSD (%)	Recovery (%)	Initial content (mg/kg)	Content after addition (mg/kg)	RSD (%)	Recovery (%)
Tyr	4.12	0.17	0.00	19.95	0.76	99.75	74.18	94.95	0.03	103.88	0.00	19.88	0.63	99.38
His	4.08	0.23	0.00	20.58	0.36	102.88	20.68	41.38	0.14	103.50	0.50	20.90	2.66	102.00
Pea	4.02	0.37	0.00	19.75	1.27	98.75	1.08	21.85	1.32	103.88	0.00	20.63	0.12	103.13
Put	4.35	0.13	0.33	20.58	1.23	101.25	117.98	137.85	1.23	99.38	0.58	20.78	0.50	101.00
Cad	4.28	0.63	0.00	19.70	1.52	98.50	39.78	60.28	1.03	102.50	0.00	20.03	0.61	100.13
Try	3.35	0.89	0.00	20.13	1.86	100.63	5.65	26.08	0.12	102.13	10.98	31.18	0.16	101.00
Agm	4.02	0.44	0.00	19.98	0.63	99.88	0.00	19.98	2.38	99.88	0.00	20.63	0.61	103.13
Spd	4.23	0.12	2.90	23.00	1.01	100.50	2.30	22.90	1.70	103.00	8.10	28.55	2.00	102.25
Spm	4.02	0.43	33.90	54.65	0.40	103.75	11.05	31.40	0.90	101.75	24.13	44.50	1.05	101.88

[a]RDS, relative standard deviation.
Level addition: 20 mg/kg of each amine for recovery testing.

rizo" (Table 3). This is coherent with the characteristic processing procedure (ripening, maturation, etc.) of this product which favours the formation of biogenic amines, whereas the heat treatment employed in processing other processed foods such as frankfurters inhibits microbial growth, the main factor responsible for amine production (Halász et al., 1994; Ruiz-Capillas & Jiménez-Colmenero, 2004a). Putrescine was the most abundant amine followed by tyramine and cadaverine, consistent with similar results observed in other studies of this type of product (Ruiz-Capillas & Jiménez-Colmenero, 2004b). However, β-phenylethylamine and spermidine levels were lower than those observed by other authors (Ruiz-Capillas & Jiménez-Colmenero, 2004b; Ruiz-Capillas et al., 2007). Frankfurters had intermediate amounts of amines with the emphasis on the level of tryptamine, apart from spermidine and spermine concentrations, which are naturally present in meat, the raw material of the developed product. In fact, these physiological amines predominated in the fresh meat while other amines were not detected. These low amounts of biogenic amines were indicative of the good hygienic quality of the meat. BA determination in these products revealed the suitability of this method for ascertaining the presence of these analytes in different matrices and with different amine levels.

In this study testing for potential interference was not performed in a specific way as the quantity of free amino acids and small peptides was unknown. Many are biogenic amine precursors and present themselves simultaneously with the targeted compounds in the samples, but are primarily eluted in the solvent front (within 2 min) since these amino acids are more polar by nature (Izquierdo-Pulido et al., 1993; Latorre-Moratalla et al., 2009; Tapia-Salazar et al., 2000; Tracy et al., 1995). Tracy et al. (1995) demonstrated the absence of interference using this cation-exchange chromatography method, thus demonstrating its high efficiency and dispensing with the no need for simple cleanup procedures.

4. Conclusion

Experimental conditions developed in this method such as column temperature, pump flow rate, mobile phase gradients and pH, permit an adequate, prompt and correct separation, identification and quantification of nine biogenic amines (tyramine, β-phenylethylamine, histamine, putrescine, cadaverine, tryptamine, agmatine, spermidine and spermine) in samples of meat and meat products. The most significant factor in this separation was the pH of the mobile phases.

This optimised and validated method provides suitable linearity, precision and high sensitivity for the simultaneous determination of nine biogenic amines in meat and meat products. This fact is of particular relevance in this type of products as these products have complex matrices as well as different composition and processing conditions which lead to a wide range of biogenic amine concentrations with different chemical structures. For this reason the versatility of this method is ideally suited for analysing this type of samples.

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IV.2. INFLUENCIA DEL CAMBIO DE COMPOSICIÓN DEL CHORIZO EN LA FORMACIÓN DE AMINAS BIÓGENAS.

IV.2.1. Konjac gel as pork backfat replacer in dry fermented sausages: Processing and quality characteristics.

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Konjac gel as pork backfat replacer in dry fermented sausages: Processing and quality characteristics

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ABSTRACT

The effect of replacing animal fat (0%, 50% and 80% of pork backfat) by an equal proportion of konjac gel, on processing and quality characteristics of reduced and low-fat dry fermented sausage was studied. Weight loss, pH, and water activity of the sausage were affected ($P<0.05$) by fat reduction and processing time. Low lipid oxidation levels were observed during processing time irrespective of the dry sausage formulation. The fat content for normal-fat (NF), reduced-fat (RF) and low-fat (LF) sausages was 29.96%, 19.69% and 13.79%, respectively. This means an energy reduction of about 14.8% for RF and 24.5% for LF. As the fat content decreases there is an increase ($P<0.05$) in hardness and chewiness and a decrease ($P<0.05$) in cohesiveness. No differences were appreciated ($P>0.05$) in the presence of microorganisms as a result of the reformulation. The sensory panel considered that NF and RF products had acceptable sensory characteristics.

1. Introduction

Dry fermented sausages are popular traditional meat products in many countries and are of great importance to the meat industry. However, these products present some negative health effects because of their high fat (25–45%) and energy content (300–450 kcal/100 g) and the fatty acid profiles of animal fat (Muguerza, Gimeno, Ansorena, & Astiasarán, 2004). There is growing evidence related to the link between the quantity and type of dietary fat and chronic disorders, such as ischemic heart disease, some types of cancer and obesity (WHO, 2003). Fat from meat is often assumed to contribute to the increase in these health problems (Ferguson, 2010; McAfee et al., 2010; Williamson, Foster, Stanner, & Buttriss, 2005) because of its relatively high contribution to fat intake. In industrialized countries, approximately 36–40% of the total calories in the food supply come from fat, nearly half of which is from meat intake (Byers, Turner, & Cross, 1993; Sheard, Wood, Nute, & Ball, 1998). Fat reduction is generally considered to be an important strategy for improving the fat content of foods, leading the meat industry to develop new formulations or modify traditional ones.

As in other meat products with similar characteristics, dry fermented sausage reformulation processes have been used to reduce fat content and/or to improve the fatty acid profile (Jiménez-Colmenero, 2007; Muguerza, Gimeno, Ansorena, & Astiasarán, 2004). However, dry fermented sausage is one of the meat products where fat reduction is most difficult. Aside from its nutritional contribution, fat contributes to the quality and acceptability properties (flavour, texture, mouthfeel, etc.) of dry sausage. On the other hand, the granulated (visible) fat also has a technological function in sausage production since it can facilitate the regular moisture release occurring during the fermentation process (Wirth, 1988). Different studies have explored the possibility of fat reduction, using reformulation strategies where the fat is replaced by lean meat (Liaros, Katsanidis, & Bloukas, 2009; Mendoza, García, Casas, & Selgas, 2001; Muguerza, Fista, Ansorena, Astiasarán, & Bloukas, 2002; Olivares, Navarro, Salvador, & Flores, 2010). In some cases this strategy is accompanied by the addition of other ingredients such as inulin (Mendoza et al., 2001), cereal and fruit fibers (García, Dominguez, Galvez, Casas, & Selgas, 2002) and short-chain fructooligosaccharides (Salazar, García, & Selgas, 2009) to produce a low calorie content and contribute to the desired product characteristics. However, this reformulation process often increases the product toughness due to higher water loss during fermentation (Muguerza et al., 2002; Olivares et al., 2010; Salazar et al., 2009). Visual differences also occur as the amount of granulated fat decreases with fat reduction. In this context, the use of konjac gels as a fat analog opens up interesting possibilities for fat reduction in this type of meat products.

Konjac glucomannan (KGM) is a neutral polysaccharide produced by the *Amorphophallus konjac*, a native plant of East Asia, where it has been used since ancient times. Among the different known fibers, KGM is of special interest because of its exceptional characteristics. It presents notable technological properties which have considerable potential for application to food technology, including in formulated foods based on myosystems (meat products). Its use as a food

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additive is authorized in Europe (E-425), and it is considered GRAS by the FDA. Konjac flour is considered a low-calorie ingredient which, given its content in non-digestible fiber, presents numerous physiological effects and therapeutic applications (Al-Ghazzewi, Khanna, Tester, & Piggott, 2007; González Canga et al., 2004; Tye, 1991; Zhang et al., 2001). Although KGM can be used for different purposes on account of its technological properties, it forms gels which combined with other ingredients (starch, carrageenates, gellan gum) can be used as 'fat analogs' in the formulation of reduced/low-fat meat products. KGM (added in different forms at various levels) has been used to reduce fat in products such as frankfurters (Jiménez-Colmenero et al., 2010; Kao & Lin, 2006; Lin & Huang, 2003; Osburn & Keeton, 2004), bologna sausage (Chin, Keeton, Longnecker, & Lamkey, 1998a,b; Chin, Keeton, Miller, Longnecker, & Lamkey, 2000), fresh sausages (Osburn & Keeton, 1994), pork nuggets (Berry & Bigner, 1996) or pâté (Delgado-Pando et al., 2011). Additionally, konjac gel, when ground down to a desired particle size can give the appearance of visible granulated fat required for use as a raw material to replace animal fats. A study of the characteristics of konjac gel fat analog compared to pork backfat and trimmed fat for use in fat reduction strategies for meat products has been reported (Jiménez-Colmenero et al., 2011). In spite of the evident potential which KGM offers in the development of fat analogs to reduce fat levels (with the additional health benefits it implies *per se*), as far as the authors are aware, no studies have been reported of its application to dry fermented sausage.

To explore these possibilities, the aim of this study was to investigate the effect of the fat reduction achieved by replacing animal fat (0%, 50% and 80% of pork backfat) with the same proportion of konjac gel, on the processing and quality characteristics of reduced and low-fat fermented dry sausage. In this paper the authors propose a different fat reduction strategy for dry sausage from those previously carried out, where the animal fat is replaced by a fat analog (konjac gel), with no change in the proportion of lean meat used.

2. Materials and methods

2.1. Materials and konjac gel preparation

Fresh post-rigor pork meat (mixture of *Musculus biceps femoris*, *M. semimembranosus*, *M. semitendinosus*, *M. gracilis* and *M. adductor*) (22.4%, 5.0%, 72.8% of protein, fat and moisture contents, respectively) and pork backfat (7.0%, 88.5% and 3.2% of protein, fat and moisture contents, respectively) were obtained from a local market and frozen at −20 °C until being used (not more than 14 days later).

Konjac gel (KG) was made with konjac flour (glucomannan 83%, 120 mesh) from Trades S.A. (Barcelona, Spain), pre-gelled cornstarch (Amigel, Julio Criado, S.L. Madrid. Spain), i-carrageenan (Hispanagar S.A, Burgos, Spain) and Ca(OH)2 (Panreac Química S.A., Barcelona, Spain). The KG preparation was based on that of Osburn and Keeton (2004) with modifications (Jiménez-Colmenero et al., 2010, 2011). Briefly, the procedure was as follows: 5% of konjac flour was homogenized (Stephan Universal Machine UM5, Stephan Machinery GmbH and Co., Hameln, Germany) with water (64.8%) for 3 min, left to rest for 5 min then homogenized further for 3 min. The i-carrageenan (1%) was then added and the mixture was homogenized again for 3 min. In a separate container the pre-gelled cornstarch powder (3%) was dispersed in water (16.2%), which was then homogenized with the previous mixture of konjac flour and i-carrageenan. This mixture was left to rest for 5 min and then homogenized further for 3 min. The mixture obtained was cooled to 10 °C, and then 10% of a Ca(OH)2 solution (1%) was added with gentle stirring at room temperature. The konjac gel was placed in suitable containers (so as to form blocks resembling native pork backfat), covered, manually overpressured to eliminate air and stored at 2 ± 2 °C until being used (within 24 h of preparation). The KG was prepared in triplicate.

Other ingredients and additives used were sodium chloride (Panreac Química, S.A. Barcelona, Spain), sodium tripolyphosphate (STP) (Manuel Riesgo, S.A. Madrid, Spain), sodium nitrite (Fulka Chemie GMBH, Buchs, Germany), and two commercial preparations ("choravi" and "curavi") (ANVISA, Arganda del Rey, Spain).

2.2. Design and manufacture of dry fermented sausage

The dry sausage was designed and formulated to obtain (by technological means) three levels of fat content, using a similar amount of lean meat (and therefore of muscle protein), since fat reduction was achieved by replacing pork backfat by the same proportion of fat analog (konjac gel). Three different formulations of dry fermented sausage (chorizo) were produced: a control sample, prepared with normal fat content (NF), using 74% meat and 18.5% pork backfat; a reduced-fat sample (RF) manufactured with 74% meat and replacing 50% of added pork backfat by the same proportion of konjac gel; and finally a low-fat sample (LF) formulated with 74% meat and replacing 80% pork backfat by the same proportion of konjac gel. All samples also contained 5.5, 0.18 and 1.85 of "choravi", "curavi" and NaCl, respectively. In these formulation conditions, the proportions of pork backfat were 18.5, 9.2 and 3.7% and those of konjac gel were 0, 9.2 and 14.8% in NF, RF and LF, respectively.

The meat and backfat were thawed before use (18 h at 2 ± 2 °C) and the sausages were produced as follows: first, the konjac gel and backfat were minced (15 mm diam. hole mincer plate) (Model FTSIII, Van Dall. S.r.l., Treviglio, Italy), and then mixed manually with the meat (1 min) and minced (15 mm) again. This batter was homogenized for 1 min (MAINCA, Granollers, Barcelona, Spain), then half of the additives (choravi, curavi and NaCl) were added and the batter was homogenized again for 1 min. Then the remaining additives were added and the mixture was homogenized for 2 mins. In all cases the final temperature was less than 11 °C. The prepared sausage mixture was immediately stuffed into collagen casing (Fibran S. A. Sant Joan de les Abadesses, Gerona, Spain) using a 4-cm diameter stuffer (MAINCA, Granollers, Barcelona, Spain). The sausages were hand-linked to standard sizes (22–23 cm), and the resultant strings of sausages were placed in a ripening cabinet (BINDER model KBF 240 Tuttlingen, Germany) programmed to operate under following conditions: 48 h at 23 °C and 90% relative humidity (RH), followed by 13 °C, 70–80% RH, until the end of the experiment (17 days). To monitor the ripening process, samples from each formulation were taken periodically for analysis.

2.3. Quality changes during ripening process

Weight loss during processing was evaluated as % of initial sample weight. Three strings of sausages for each formulation were used for these determinations.

The pH was determined using a pH meter (model 827pH Lab Methrom, Herisau, Switzerland) on 10 g homogenate samples in 100 ml of distilled water. Three measurements were performed for each sample.

Water activity (a_w) during processing was measured at 25 °C with a LabMaster-aw (Novasina, Lachen, Switzerland). Three determinations were carried out for each sample.

Oxidative stability was evaluated by changes in thiobarbituric acid-reactive substances (TBARS). The TBARS measurement procedure was based on methods used by Serrano, Cofrades, and Jiménez-Colmenero (2006). Briefly, the procedure was as follows: 5 g of each sample was homogenized in 35 ml of 7.5% trichloroacetic acid for 30 s in a high speed Ultraturrax blender (Ika-Werke, GmbH & Co, Staufen, Germany). The sample was centrifuged (3000 *g*, 2 min) and 5 ml of the supernatant was mixed with 5 ml of 20 mM

thiobarbituric acid; finally the solution was mixed and kept in the dark for 20 h at 20 ± 1.5 °C. The pink color formed was determined spectrophotometrically (Lambda 15UV/VIS spectrophotometer, Perkin-Elmer, USA) at 532 nm. A calibration curve was plotted with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO, USA) to obtain the malonaldehyde (MDA) concentration and results were expressed as mg malonaldehyde/kg of sample. The TBARS determinations were performed in triplicate.

Residual nitrite content during processing was determined using the flow injection analysis (FIA) technique according to Ruiz-Capillas, Aller-Guiote, and Jiménez-Colmenero (2007). Briefly, at least two extracts were prepared from each sample and injected into the FIA equipment using ammonium chloride as first reagent. The nitrite determination was based on its reaction with sulphanilamide to form a diazonium salt which was coupled with N-(1-Naphthyl)-ethylendiamino-dihydrochloid monomethanolat (NED) to yield an azo dye compound and its absorbance was spectrophotometrically determined at 540 nm. Results are averages of at least 6 determinations.

2.4. Proximate analysis and quality characteristics of dry fermented sausages

Sample moisture and ash contents were determined (AOAC, 2005) in triplicate in all samples. Protein content was measured in quadruplicate with a LECO FP-2000 nitrogen determinator (Leco Corporation, St Joseph, MI, USA). Fat content was evaluated in triplicate according to Bligh and Dyer (1959). Carbohydrates were calculated taking into account the ingredient composition and formulation content, as well as water loss during ripening. Energy values were estimated from protein (×4 kcal/g), carbohydrate (×4 kcal/g) and fat (×9 kcal/g) contents for each product.

Color, CIE-LAB tristimulus values, lightness, L* and redness, a* of samples were evaluated on a Chroma Meter CR-400 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). Yellowness was not studied since it has a minor effect on sausage color. The determinations were carried out on 2 cm cross-sections of recently cut sausage. Fifteen determinations were performed from each formulation.

Texture profile analysis (TPA), as described by Bourne (1978), was carried out using a TA.XT2i Stable Micro Systems Texture Analyser (Stable Microsystems Ltd., Surrey, England). Ten cores (diam = 20 mm, height = 20 mm) per sample were axially compressed to 50% of their original height. A 30-kg load cell was used at a crosshead speed of 1 mm/s. Attributes were calculated as follows: hardness (Hd) = peak force (N) required for first compression; cohesiveness (Ch) = ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp) = distance (mm) the sample recovers after the first compression; chewiness (Cw) = Hd × Ch × Sp (N × mm). Measurements were performed at 22 °C.

Microbiological analysis of the dry fermented sausage was carried out as follows: 10 g of each sample (from 2 sausages) was taken and placed in a sterile plastic bag with 90 ml peptone water (0.1%) with 0.85% NaCl. After 2 min in a stomacher blender (Stomacher Colworth 400, Seward, UK), appropriate decimal dilutions were pour-plated (1 mL) on the following media: Plate Count Agar (PCA) (Merck, Germany) for the total viable count (TVC) (30 °C for 72 h); De Man, Rogosa, Sharp Agar (MRS) (Merck, Germany) for lactic acid bacteria (30 °C for 3–5 days); and Violet Red Bile Glucose Agar (VRBG) (Merck, Germany) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of colony-forming units per gram (log cfu/g).

The sensory analysis of the final products was performed by 16 panelists previously trained with two training sessions in the products and terminology (Standard UNE 87-001-94) using commercial fermented sausage. The samples were presented to the panelists in oblique 2 mm thick slices. A hedonic scale (anchored at both ends) rating test was carried out where the testers evaluated the following for each sample: appearance (0 = not characteristic, 10 = characteristic), flavor (0 = very mild, 10 = very strong), Firmness (0 = very soft, 10 = very hard), juiciness (0 = very dry, 10 = very juicy) and overall acceptability (0 = dislike extremely, 10 = like extremely). The evaluation was made on a non-structured scale with fixed extremes. Each point was later converted to a numerical scale.

2.5. Statistical analysis

A two-way analysis of variance (ANOVA) was carried out depending on the variables: type of dry fermented sausage and ripening time. One-way ANOVA was also carried out to evaluate the statistical significance ($P<0.05$) in the quality characteristics of the different fermented sausages (NF, RF, LF). Tukey's HSD test was used to identify significant ($P<0.05$) differences between types of fermented sausage and time, and least squares differences for comparison of mean values between different sausages. Pearson product–moment correlation (R) was performed to assess the relationships between variables. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, U.S.A.). The experiment was replicated twice.

3. Results and discussion

3.1. Processing characteristics

As a result of the drying process, the processing time increased weight loss in all sausages (Fig. 1). The greatest weight loss ($P<0.05$) occurred as the fat percentage in the sausage was reduced. This result is related to the higher water content in the original mixture, since in the formulation strategy animal fat (backfat) was replaced by konjac gel, which contains around 94% water. This loss occurs even though the water is integrated into the konjac gel structure and this ingredient with the appropriate grain size forms part of the meat matrix. In agreement with the results of this experiment, other authors have also reported an inverse ratio between weight loss and fat content during the dry fermented sausage drying process.

The pH evolution in all formulations was similar to that of conventional dry fermented sausages (Fig. 2). The initial pH of the fermented sausages, ranging from 5.89 to 6.06, was affected ($P<0.05$) by fat levels. This result may be obtained because the konjac gels have a higher pH than the fat they are replacing. In all samples the initial pH decreased quickly during the first few days, so that by the end of processing the three types of sausage presented similar pH values ($P>0.05$) of 4.80–4.88 (Fig. 2); these pH values agree with those reported for commercial dry fermented sausages (Herrero et al., 2007). Changes in pH such as those observed in this experiment during dry fermented sausage processing, due to the increased lactic acid content from the effect of microbial activity, have been reported by

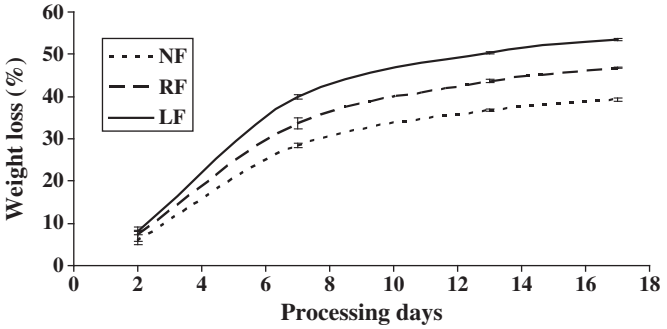

Fig. 1. Weight loss (%) during processing of normal fat (NF), reduced fat (RF) and low-fat (LF) dry fermented sausage. Standard deviations are represented by vertical intervals crossing the curve lines.

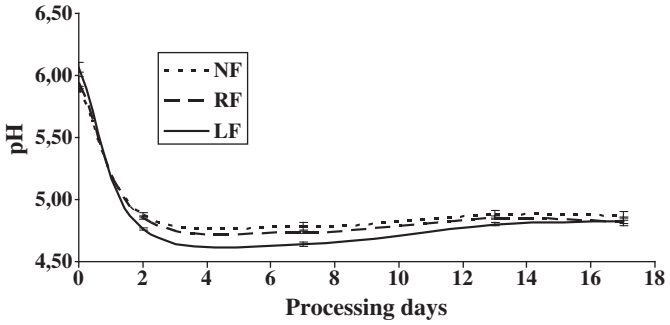

Fig. 2. Changes in pH values of normal fat (NF), reduced fat (RF) and low-fat (LF) dry fermented sausage as affected by processing. Standard deviations are represented by vertical intervals crossing the curve lines.

other authors (García et al., 2002; Mendoza et al., 2001; Muguerza et al., 2002; Salazar et al., 2009).

The water activity of the sausages was affected ($P<0.05$) by fat reduction and processing time, with no significant interaction between the two variables. As a result of the drying process and the corresponding salt concentration, a_w decreased ($P<0.05$) from initial values of 0.95–0.97 in the original mixture to 0.81–0.83 at the end of the ripening period (Fig. 3). While initially the NF sample (0% konjac gel) presented the lowest ($P<0.05$) value for a_w, at the end of the ripening period the lowest ($P<0.05$) a_w value was observed for the product with the highest percentage of konjac gel (LF), which was the one which showed the greatest weight loss (Fig. 1). Similar a_w levels for dry fermented sausage have been reported (García et al., 2002; Mendoza et al., 2001; Salazar et al., 2009). A correlation (0.897, $P<0.001$) was established between a_w and weight loss.

The lipid oxidation (TBARS values) of the sausages was not affected ($P>0.05$), by fat reduction (konjac gel proportion), but was affected ($P<0.05$) by processing time. Irrespective of the formulation, lipid oxidation increased ($P<0.05$) during ripening from initial TBARS values of 0.19 mg MDA/kg to values of 0.88 mg MDA/kg at the end of the ripening period. Although the rate and extension of lipid oxidation are generally favored by fat content, this effect was not observed in this study. This may be related to the low lipid oxidation rate and levels observed in the dry sausages in this experiment. TBARS values ranging from 0.5 to 2 mg MDA/kg have also been reported in dry fermented sausage (Ansorena & Astiasarán, 2004; Liaros et al., 2009).

Although initially the residual nitrite levels were affected by formulation (NF, 47.3 ± 1.58; RF, 49.9 ± 1.43; LF, 61.9 ± 1.09 mg/kg), by the end of the ripening period all sausages had similar ($P>0.05$) levels of residual nitrite (2.6 ± 0.07). Initial differences between the sausages seem to be related more to fat levels than to the presence of konjac gel. Nitrite is a reactive chemical which reacts (with different chemical constituents) when it is added to the meat system, and so a reduction of the residual nitrite is detected in the product (Cassens, Greaser, Ito, & Lee, 1979). Some of these constituents (e.g. lipids) are present in the original reformulated sausage mixture (RF and LF) in lower proportion than in the NF sausage due to the reformulation strategy (replacing animal fat with konjac gel). Higher levels of residual nitrite were observed in these samples as less nitrite reacts with the lipids. However, the reactivity of the nitrite in the meat matrix means that this effect is not appreciable at the end of the ripening period. Although the authors are not aware of studies of residual nitrite in dry-fermented sausages, in cooked meat products an inverse relationship between residual nitrite and fat level of the product has been reported (Jiménez-Colmenero, Carballo, Fernández, Cofrades, & Cortés, 1997; Jiménez-Colmenero et al., 2010). It has also been reported that the addition of konjac to wiener formulation (Kilic, Cassens, & Borchert, 2002) or to frankfurters (Jiménez-Colmenero et al., 2010) does not influence the residual nitrite in the finished product. It has also been reported that as a result of the processing time, nitrite levels decreased from initial values of 27.8 to 6.7 and 40–48 to 5–15 mg/kg in Turkish dry fermented sausage and Spanish dry fermented sausage, respectively (Ercoskun & Özkal, 2011; Fernández-López, Sendra, Sayas-Barberá, Navarro, & Pérez-Alvarez, 2008). Fernández-López et al. (2008) reported that this effect ocurred during fermentation, but not during the drying-curing stage.

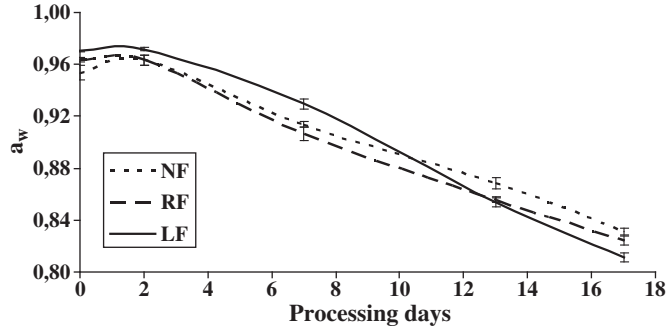

Fig. 3. Water (a_w) activity of normal fat (NF), reduced fat (RF) and low-fat (LF) dry fermented sausage as affected by processing. Standard deviations are represented by vertical intervals crossing the curve lines.

3.2. Proximate analysis and energy content

As expected, the partial replacement of pork backfat by konjac gel affected the proximate composition of the dry fermented sausage (Table 1). Fat content was affected ($P<0.05$) by product formulation. The fat content of the normal fat (NF) sausage was 29.96%, similar to that of conventional sausage (Mendoza et al., 2001). In reduced-fat (RF) and low-fat (LF) sausages, the fat levels were 19.69% and 13.79%, respectively. These changes represent a fat reduction of around 34% (RF) and 54% (LF). In the rest of the constituents, as the fat content is reduced increases were observed in the proportion of moisture, protein, ash and carbohydrates ($P<0.05$). These changes (quantitatively low as compared with fat variation) in the levels of protein, ash and to a lesser extent carbohydrates (from the effect of the presence of konjac gel), are basically attributable to the differences in weight loss between different formulations during ripening. Less effect can be attributed to the strategy formulation since the only difference between the different sausage preparations was the partial replacement of animal fat by konjac gel. When other formulation strategies are used, such as the replacement of animal fat (backfat) by lean meat, with or without the addition of other ingredients (García et al., 2002; Liaros et al., 2009; Mendoza et al., 2001; Muguerza et al., 2002; Olivares et al., 2010; Salazar et al., 2009), the contributions of both factors to the proximate composition are different. In these conditions, different levels of fat reduction have been

Table 1
Proximate analysis and energy values of normal fat (NF), reduced fat (RF) and low fat (LF) dry fermented sausage.

	NF	RF	LF
Moisture (%)	31.87 ± 0.73^a	33.32 ± 0.27^b	35.10 ± 0.28^c
Protein (%)	25.64 ± 0.13^a	30.37 ± 0.81^b	32.52 ± 0.44^b
Fat (%)	29.96 ± 0.49^c	19.69 ± 0.41^b	13.79 ± 0.48^a
Ash (%)	6.51 ± 0.05^a	7.60 ± 0.04^b	8.14 ± 0.04^c
Carbohydrates (%)	3.92	6.03	7.98
Energy value (kcal/100 g)	387.92 ± 4.75^c	322.78 ± 0.44^b	286.12 ± 3.84^a
Calories from fat (kcal/100 g)	269.67 ± 4.45^c	177.19 ± 3.65^b	124.13 ± 4.30^a
Calories from fat (%)	71.1	54.9	42.7
Fat reduction (%)	–	34.3	54.0
Energy value reduction (%)	–	14.8	24.5

Means ± standard deviation. Different letters in the same row indicates significant differences ($P<0.05$).

reported, in some cases of up to 60% (García et al., 2002; Mendoza et al., 2001; Muguerza et al., 2002; Salazar et al., 2009).

The energy values of the dry fermented sausage decreased ($P<0.05$) as fat content decreased (Table 1). The fat contribution to the energy varied from 71.1% of the total fat-derived caloric value in NF, to 54.9% in RF and 42.7% in LF sample. These changes represent an energy reduction of around 14.8% for RF and 24.5% for LF samples. Different proportions of total energy reduction (up to 35%) have been reported as the result of fat reduction in dry fermented sausage (García et al., 2002; Mendoza et al., 2001; Salazar et al., 2009).

In accordance with European Union labeling regulation N° 1924/2006 (EC, 2007), the LF product can be included in the "reduced-fat" product category since the reduction in fat content is over 30% compared to the conventional product.

3.3. Quality characteristics of sausages

3.3.1 . Color

While lightness was not affected ($P>0.05$) with partial replacement of pork backfat by konjac gel (fat reduction), redness tended to decrease, although this effect was significant only for the LF sample (Table 2). These small differences may be related to the effects of both the fat reduction and added konjac gel. Other studies have shown that when fat content is reduced, the product is darker and redder than a high-fat product. This effect was observed in cooked sausage (Carballo, Fernández, Barreto, Solas, & Jiménez-Colmenero, 1996) and dry fermented sausage (Muguerza et al., 2004; Salazar et al., 2009). However, Muguerza et al. (2002) reported that the redness of dry fermented sausage was not affected by fat level, while high fat content resulted in lighter sausage. It is generally accepted that the red color of fermented sausages develops as the result of the reaction of nitric oxide, produced from nitrites, with myoglobin producing nitrosylmyoglobin. As a result, variations in the myoglobin concentration (among other factors such as pH, weight loss during processing, etc.), linked with changes in composition (related to fat reduction strategy), may affect the product color (Liaros et al., 2009). In this context, the fat reduction processes are accompanied by an increase in the myoglobin concentration in the meat system when the fat is reduced by replacing backfat by lean meat. However, the change in the concentration is very small when a fat analog is used as a substitute for the backfat (with the lean meat remaining constant) as is the case in this experiment.

Table 2
Color, TPA parameters, microbial counts and sensorial evaluation of normal fat (NF), reduced fat (RF) and low fat (LF) dry fermented sausage.

		NF	RF	LF
Colour	Lightness (L*)	51.43 ± 1.99^{a}	51.52 ± 1.86^{a}	50.01 ± 0.76^{a}
	Redness (a*)	13.40 ± 1.01^{b}	13.15 ± 1.14^{b}	11.83 ± 1.01^{a}
TPA parameters	Hardness (N)	50.59 ± 7.17^{a}	74.63 ± 9.23^{b}	133.47 ± 8.19^{c}
	Cohesiveness (dimensionless)	0.46 ± 0.04^{c}	0.41 ± 0.05^{b}	0.38 ± 0.04^{a}
	Springiness (mm)	0.52 ± 0.04^{ab}	0.50 ± 0.03^{a}	0.58 ± 0.07^{b}
	Chewiness (N × mm)	11.94 ± 2.16^{a}	15.67 ± 3.37^{b}	31.11 ± 2.90^{c}
Microbial counts (log cfu/g)	Total viable count	8.71 ± 0.05^{a}	8.78 ± 0.00^{a}	8.94 ± 0.14^{a}
	Lactic acid bacteria	9.00 ± 0.00^{a}	8.91 ± 0.12^{a}	9.05 ± 0.14^{a}
Sensorial evaluation	Appearance	7.69 ± 1.69^{a}	7.53 ± 1.66^{a}	6.48 ± 1.98^{a}
	Flavor	5.42 ± 1.35^{a}	5.31 ± 1.55^{a}	5.49 ± 2.07^{a}
	Firmness	4.07 ± 1.32^{a}	6.08 ± 1.59^{b}	7.81 ± 1.10^{c}
	Juiciness	6.95 ± 1.43^{b}	5.16 ± 2.19^{b}	2.76 ± 1.88^{a}
	Overall acceptability	6.82 ± 1.93^{b}	6.49 ± 1.55^{b}	4.03 ± 2.26^{a}

Means ± standard deviation. Different letters in the same row indicates significant differences ($P<0.05$).

The effect of konjac gel on the color parameter of sausages seems to be very limited as the color of pork backfat is relatively similar to that of konjac gel. This means that although the fat analog (with similar grain size) had lower L* and a* values than backfat, these differences are quantitatively small. (Jiménez-Colmenero et al., 2011). It has been reported that when compared with full-fat frankfurters, in reduced-fat products L* generally tended to decrease and a* to increase with the addition of konjac gel (Jiménez-Colmenero et al., 2010; Kao & Lin, 2006; Lin & Huang, 2003). Similar effects have been reported in low-fat pork sausages (Osburn & Keeton, 1994). This effect was not observed in this experiment, probably due to differences in the nature and processing conditions of both types of meat products, as commented previously.

3.3.2 . Texture

TPA parameter results are shown in Table 2. It can be observed that the decrease in the fat content (and increase in the konjac gel level) produces an increase ($P<0.05$) in hardness, and chewiness, probably due to the percentage weight loss (Fig. 1). A correlation (0.880, $P<0.001$) was established between hardness and weight loss. Partial replacement of animal fat by konjac gel decreased ($P<0.05$) cohesiveness, while no clear effect was observed on springiness (Table 2). These results may be related to the effects of both fat reduction and added konjac gel. The formation of harder structures has been reported as fat content decreases in dry fermented sausages (Liaros et al., 2009; Muguerza et al., 2002, 2004), and in cooked meat products (Carballo et al., 1996). These findings are consistent with the results of this experiment, even although different fat reduction formulation strategies were used. Konjac gel has been used to simulate fat characteristics and reduce fat in different cooked meat products (Jiménez-Colmenero et al., 2010). It has been observed that in these gel/emulsion meat products the effect of replacing pork backfat with konjac gels varies according to the nature of the konjac gel and the proportion of fat replaced (Jiménez-Colmenero et al., 2010; Kao & Lin, 2006; Lin & Huang, 2003; Osburn & Keeton, 2004). However, it does not seem appropriate to extrapolate this behavior to what may occur when it is included in dry fermented sausage as in this study. This is because the contribution of the konjac gel to the product structure seems clearly different. While in gel/emulsion type products (e.g. frankfurter, bologna), the konjac gel presents a high level of (comminuted) structural disintegration and is therefore integrated into a very homogeneous (cooked) protein matrix, in our dry fermented sausage the konjac gel presents a larger particle size as it is in granulated (visible fat-like) form embedded in an uncooked and more heterogenous meat matrix.

3.3.3 . Microbiology

No differences were observed ($P>0.05$) in the presence of total viable aerobes and lactic acid bacteria in dry fermented sausages from the effect of reformulation (Table 2). The levels of microorganisms detected (including enterobacteriaceae<4 log cfu/g) are within the normal range observed in this type of product, with lactic bacteria as the dominant flora (Bloukas, Paneras, & Fournitzis, 1997; González & Díez, 2002; González-Fernández, Santos, Jaime, & Rovira, 2003; González-Fernández, Santos, Rovira, & Jaime, 2006; Liaros et al., 2009). High levels of lactic bacteria in foodstuffs have been related to positive effects on human health (Muguerza et al., 2002). The ripening process of the samples are subjected to facilitate the active growth of microorganisms, mainly lactic bacteria, producing a rapid fall in pH (Fig. 2). In agreement with the results of this experiment various authors (Bloukas et al., 1997; Muguerza et al., 2002) did not observe variations in microbial growth in fermented sausage depending on the replacement of the pork backfat by oil-in-water emulsion. No differences in lactic acid bacteria and enterobacteriaceae counts were observed between the high-fat and low-fat control fermented sausages, although in this case the fat

was reduced by an increase in lean meat (Koutsopoulos, Koutsimanis, & Bloukas, 2008; Liaros et al., 2009). Similarly, no differences were observed in the microbial growth from the effect of the inclusion of fiber in dry fermented sausages with different fat levels (Fernández-López et al., 2008; Salazar et al., 2009). In other types of product such as fresh pork low-fat sausages and lamb, heating low-fat sausages made with konjac gels, Osburn and Keeton (1994, 2004) did not observe changes either in the microbial growth.

3.3.4. Sensory evaluation

Table 2 shows the sensory scores obtained for dry fermented sausages. No differences ($P<0.05$) were found in appearance and flavor between the three samples. The flavor of fermented sausages is influenced by several factors, primarily the source, quantity and type of ingredients, but also by processing conditions. The main difference between products is related to the fat level, and since the products still retain important levels of animal fat, the variations in flavor are not appreciable. Liaros et al. (2009) reported no differences in flavor affected by fat level in fermented sausages. Similar cross section appearance indicates that konjac gel in the meat matrix presents a visual aspect close to the fat it is replacing; other authors report that fat reduction decreases the appearance of dry fermented sausage (Liaros et al., 2009; Muguerza et al., 2002). According to instrumental hardness measurements, as the fat content decreases (and konjac gel levels increase) an increase ($P<0.05$) in firmness occurs. Juiciness and general acceptability showed similar behavior. The lowest ($P<0.05$) scores were found for LF samples, while no differences ($P>0.05$) were observed between NF and RF samples. Lower juiciness values could be partly related to greater weight loss during processing. Since the difference between the juiciness of the samples does not seem to be linked to the moisture level (Table 1), the level of water release in chewing may be a determinant factor. The LF sample is formulated with a higher proportion of konjac gel, which contains high water levels. Better water binding properties of konjac gel as compared with muscle structure (along with a lower fat level), release less fluids on chewing, with a resultant reduction in juiciness. In agreement with our results, Mendoza et al. (2001) observed that the main effects of fat reduction in dry fermented sausage were related to decreased juiciness and tenderness.

Few studies have reported the effect of the addition of konjac to meat products. No noticeable changes in sensory quality were observed in frankfurters as a result of fat reduction and substitution of konjac gel for pork backfat (Jiménez-Colmenero et al., 2010). Osburn and Keeton (2004) reported that low-fat sausages with konjac gel slightly reduced sensory panel values.

Overall, the panel considered that NF and RF products had acceptable sensory characteristics. However the main factors which limit the general acceptability of the LF sample are associated with the formation of harder and dried meat systems.

In conclusion, partial replacement of pork backfat with konjac gel opens up new possibilities for fat reduction in dry fermented sausage. The design of healthier meat products in which fat content is reduced and konjac glucomannan is presented is a promising avenue for research. Depending on the level of substitution (fat reduction), this reformulation strategy presents different effects on processing and quality characteristics. High levels of animal fat replacement by konjac gel may present undesirable results in weight loss, hardness and juiciness, which would have to be limited to boost the use of this technology. To this end, two proposals can be considered: the first is to modify the processing conditions. Variations in processing conditions have been used as an effective technique to improve processing and quality characteristics of dry fermented sausage production in fat reduction processes (Liaros et al., 2009; Olivares et al., 2010). The second possibility is to modify the properties of the konjac gel used as a fat analog, improving its water-binding properties to hinder evaporation in the drying process. If the behavior of the other components of the meat system remains unchanged, this would reduce weight loss and hardness and improve juiciness.

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IV.2.2. Biogenic amine formation in low- and reduced-fat dry fermented sausages formulated with konjac gel.

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Biogenic Amines in Low- and Reduced-Fat Dry Fermented Sausages Formulated with Konjac Gel

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ABSTRACT: Biogenic amines in low- and reduced-fat dry fermented sausages made with konjac gel (KG) as pork backfat replacer were studied. An increase ($P < 0.05$) was observed in the microbial count during the fermentation process, reaching levels of over 8 Log cfu/g of total viable microorganisms and lactic acid bacteria. However, no significant differences were observed in the microbiota evolution as a function of the reformulation process (fat and konjac gel content). High levels of physiological amines (spermidine, spermine, and agmatine) were observed in the raw material. From day 2 of the fermentation process an increase ($P < 0.05$) was observed in tyramine and putrescine, which were the predominant amines at the end of the storage period. The increase in these amines was proportional to the presence of KG and fat reduction. This can also be seen for spermine, with agmatine showing the inverse. The biogenic amine levels in these products reformulated with KG are not considered to pose a health risk to consumers.

KEYWORDS: *konjac gel, dry fermented chorizo sausages, microbiology, biogenic amines*

INTRODUCTION

Biogenic amines are formed widely in fermented products (including fermented meat products), mainly by decarboxylation of free amino acids by the action of enzymes of microbiological origin. The biogenic amine content depends on a number of interrelated factors such as the raw material (meat composition, pH, handling and hygienic conditions, etc.), additives (salt, sugar, nitrites, etc.) affecting free amino acid availability, microbiological aspects (bacterial species and strain, bacterial growth, etc.), technical processing of the meat or meat products (e.g., steaks, roasts and hams, and ground, restructured, comminuted, fresh, cooked, smoked, and fermented meats, etc.), and storage conditions (time/temperature, packaging, temperature abuse, etc.). The combined action of all these factors will determine the final biogenic amine profile and concentrations by directly or indirectly determining substrate and enzyme presence and activity.[1−3] Fermented products are among the meat derivates which generally have the highest levels of biogenic amines,[4−7] since the production process is led by microorganisms which may contain amino acid decarboxylase.[8,9] The leading bacterial group during the sausage ripening process is lactic acid bacteria, which constitute the predominant microbiota during most of the process, and lactic acid bacteria are the main producer of biogenic amines.[8−12] The presence of biogenic amines in fermented products is a health and quality concern; therefore, there is evidently interest in the control and reduction of the amounts of biogenic amines in meat derivates and foodstuffs in general.

Like other agrifood sectors, the meat industry is undergoing major transformations, driven among other things by changes in consumer demands, leading to development of healthier meat products. In this context, lipids are among the components that have received the most attention in relation to development of these kinds of meat products.[12] This is especially important in products like dry fermented sausages, popular traditional meat products, which have some negative health concerns because of their high-fat (25−45%) and energetic content (300−450 kcal/100 g), and animal fat fatty acid profiles.[13] This is why fat reduction is generally considered as an important strategy to improve fat content, leading to new or modified traditional formulations.

Different studies have explored the possibilities of fat reduction in dry fermented sausages using fat replacement by lean meat as a formulation strategy, often linked to addition of nonmeat ingredients.[13−16] However, these reformulation processes often increase product toughness due to higher water losses during fermentation. In a previous work,[17] our research group studied a fat reduction strategy in dry fermented sausages based on replacing animal fat by konjac gel (without altering the proportion of lean meat). This study, through an evaluation of the processing and quality characteristics of reduced- and low-fat fermented dry sausage, demonstrated the viability of this reformulation strategy, depending on fat reduction through replacement of animal fat, showing that the reformulated products had acceptable sensory characteristics. With this reformulation strategy, as well as the health effects associated with fat reduction, there are other effects of the presence of konjac gel, including numerous physiological effects and therapeutic applications.[18−20] It has been suggested that fiber-like KGM exert chemoprotective effects in the colonocytes which may prevent the occurrence of colorectal tumors.[21] Although konjac gel has been used in reformulation of various meat products such as frankfurter, bologna, fresh sausages, chicken nuggets, or pâté,[22−28] to our knowledge, no

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other studies have been reported of its application to dry fermented sausage, apart from the one focusing on the technological and sensorial properties carried out by this research group.[17]

Changes in formulation, processing, and preservation conditions can obviously alter various factors (e.g., presence of free amino acids, decarboxylase enzyme content, and suitable environmental conditions), which may potentially affect formation of biogenic amines in quantitative and qualitative terms.[1] This effect has been described in meat product reformulation processes based on incorporation of ingredients such as seaweed or walnut.[29,30] As a result, it would not be appropriate to approach development of healthier products without taking into account these considerations, which may compromise the safety of these products. This is particularly important in dry fermented sausages where, as mentioned above, the presence of biogenic amines is particularly relevant because of their composition and processing.

On this basis, the aim of this paper was to evaluate how compositional changes, associated with the reformulation processes for a new, healthier formulation of dry fermented sausage, can affect the quantitative and qualitative levels of biogenic amines. To do this, a study was carried out on the effect of replacing animal fat (0%, 50%, and 80% of pork backfat) by an equal proportion of konjac gel on formation of biogenic amines and microbial growth during the fermentation and ripening stages in the processing of reduced- and low-fat fermented dry sausage.

MATERIALS AND METHODS

Materials, Konjac Gel, and Dry Fermented Sausage Preparation (Chorizo). Raw meat materials and additives as well as konjac gel (KG) and dry fermented preparation have been reported by Ruiz-Capillas et al.[17] Briefly, fresh postrigor pork meat and pork backfat were obtained from a local market. The KG was prepared in triplicate as follows: 5% konjac flour (glucomannan 83%, 120 mesh) from Trades S.A. (Barcelona, Spain) was homogenized (Stephan Universal Machine UM5, Stephan Machinery GmbH and Co., Hameln, Germany) with water (64.8%) for 3 min, left to rest for 5 min, and then homogenized for a further 3 min, i-carrageenan (1%) (Hispanagar S.A, Burgos, Spain) was then added, and the mixture was homogenized again for 3 min. Pregelled cornstarch powder (3%) (Amigel, Julio Criado, S.L. Madrid, Spain) was dispersed in 16.2% of water and homogenized with a mixture of konjac flour and i-carrageenan, left to rest for 5 min, and then homogenized for a further 3 min. The mixture was cooled to 10 °C, and then 10% of a $Ca(OH)_2$ solution (1%) was added with gentle stirring at room temperature. Konjac gel was placed in suitable containers (so as to form blocks resembling native pork backfat) and stored at 2 ± 2 °C until used (within 24 h of preparation).

Three different formulations of dry fermented sausage (chorizo) were produced: a control sample, prepared with normal fat content (NF), using 74% meat and 18.5% pork backfat; a reduced-fat sample (RF) prepared with 74% meat and replacing 50% of added pork backfat by the same proportion of konjac gel; and, finally, another low-fat sample (LF) formulated with 74% meat and replacing 80% pork backfat by the same proportion of konjac gel. All samples also contained 5.5%, 0.18%, and 1.85% of the two commercial curing salts "choravi", "curavi" (ANVISA, Arganda del Rey, Spain), and NaCl, respectively. In these formulation conditions the pork backfat proportions were 18.5%, 9.2%, and 3.7%, and the konjac gel proportions were 0%, 9.2%, and 14.8% in NF, RF, and LF, respectively. No starter culture was used. Preparation of this chorizo-type dry fermented sausage was reported in detail by Ruiz-Capillas et al.[17] Briefly, the konjac gel and backfat were minced at 15 mm (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy) and then mixed manually with the meat (1 min) and minced at 15 mm again. This batter was homogenized with the additives ("choravi", "curavi", and NaCl). In all cases the final temperature was less than 11 °C. The prepared sausage mixture was stuffed into collagen casing (Fibran S. A. Sant Joan de les Abadesses, Gerona, Spain) using a 4 cm diameter stuffer (MAINCA, Granollers, Barcelona, Spain), and the sausages were placed in a ripening cabinet (BINDER model KBF 240 Tuttlingen, Germany) programmed to operate under following conditions: one fermentation step for 48 h at 23 °C and a relative humidity (RH) of 90% followed by another ripening process step at 13 °C, 70−80% RH, until the end of the experiment (17 days). To monitor the ripening process, samples from each formulation were taken periodically for analysis.

Proximate Analysis of Dry Fermented Sausages. Sample moisture and ash contents were determined[31] in triplicate in all samples. Protein content was measured in quadruplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corp., St. Joseph, MI). Fat content was evaluated in triplicate according to Bligh and Dyer.[32] Carbohydrates were calculated taking into account ingredient composition (konjac flour, i-carrageenan, and cornstarch plus the curing salts "choravi") and amount used.[17]

Microbiological Analysis. Microbiological analysis of sausages samples during processing (fermented and ripening steps) was carried out as follows: 10 g of each sample (from 2 sausages) was taken and placed in a sterile plastic bag with 90 mL of peptone water (0.1%) with 0.85% NaCl. After 2 min in a stomacher blender (Stomacher Colworth 400, Seward, U.K.), appropriate decimal dilutions were pour plated (1 mL) on the following media: Plate Count Agar (PCA) (Merck, Germany) for the total viable count (TVC) (30 °C for 72 h); De Man, Rogosa, Sharp Agar (MRS) (Merck, Germany) for lactic acid bacteria (30 °C for 3−5 days); and Violet Red Bile Glucose Agar (VRBG) (Merck, Germany) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of colony-forming units per gram (Log cfu/g).

Analysis of Biogenic Amines by Ion-Exchange Chromatography. Tyramine, phenylethylamine, histamine, putrescine, cadaverine, agmatine, triptamine, spermidine, and spermine were determined in an extract prepared by blending 25 g of each sample (two sausages were taken from sample) with 50 mL of 7.5% trichloroacetic acid in an omnimixer (Omni Internacional, Waterbury, CT) (20000 rpm, 3 min) and centrifuged at 5000*g* for 15 min at 4 °C in a desktop centrifuge (Sorvall RTB6000B, DuPont, USA). Supernatants were filtered through a Whatman no. 1 filter and passed back through a 0.22 μm Nylon filter (Millipore, Ireland). This filtrate was injected into an HPLC model 1022 with a Pickering PCX 3100 postcolumn system (Pickering Laboratories, Mountain View, CA) following the methodology described by Triki et al.[33] The results are the mean of at least 3 determinations.

Statistical Analysis. In the processing determinations two-way analyses of variance (ANOVA) as a function of type of fermented sausage (NF, RF, LF) and processing time were performed. Tukey's HSD test was used to identify significant ($P < 0.05$) differences between type of fermented sausage and time and least-squares differences for comparison of mean values between different sausages. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL). The experiment was replicated twice.

RESULTS AND DISCUSSION

Proximate Analysis. The proximate composition of dry fermented sausages (Figure 1) was affected ($P < 0.05$) by formulation (partial replacement of pork backfat with KG) as described previously by Ruiz-Capillas et al.[17] In the reduced-fat (RF) and low-fat (LF) sausages, the fat levels were 19.69% and 13.79%, respectively, compared with 29.96% of the batch with normal fat (NF, control). Under these experimental conditions a general trend was observed that as the fat content is reduced the proportion of moisture, protein, ash, and carbohydrates increases (Figure 1).

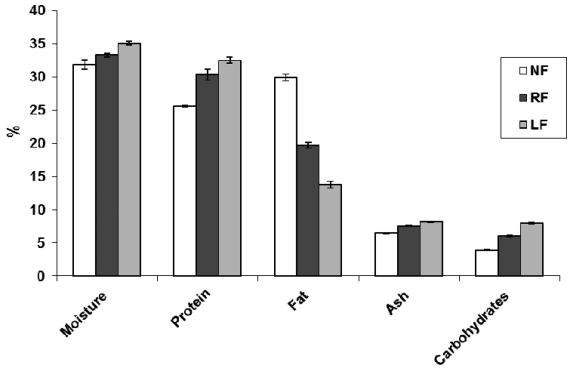

Figure 1. Proximate analysis (%) of dry fermented sausage chorizo samples with different levels of fat (normal fat (NF), reduced fat (RF), and low fat (LF)). Tool bars indicate the standard error of the individual data.

Microbiology Analysis. The results of microbiology counts performed on the different products during the manufacturing process are shown in Table 1. The initial levels (in raw material) of total viable micro-organisms and lactic acid bacteria were 6.34−6.72 and 4.54−4.69 Log cfu/g, respectively, being lower in sausages with added konjac gel. After day 2 of processing, during the fermentation period, a sharp and significant increase was observed (two logarithmic units) in the microbial population, reaching levels higher ($P < 0.05$) than 8 and 6 Log cfu/g in the total viable and lactic acid bacteria, respectively (Table 1). This increase was produced mainly by the fermentation process conditions (48 h at 23 °C RH 90%, see Materials and Methods), which favor growth of the micro-organisms, mainly lactic acid bacteria, with a corresponding rapid decrease in pH,[17] due to the metabolic activity of these bacteria. A similar behavior of lactic acid bacteria has been described in other studies on the processing of chorizo.[34]

On day 7 of the process, during ripening, an increase in the level of micro-organisms was observed, significant in the case of the lactic acid bacteria which became (quantitatively) the predominant flora. These levels were maintained without significant changes until the end of processing. The lower growth rate of the microbiota in this second phase of the chorizo processing may be attributed to the ripening process conditions (13 °C, RH 70−80), as indicated in a previous study.[17] This evolution has also been reported in other studies of dry chorizo sausages.[34,35] Other authors have pointed out that the temperature at which fermentation takes place (usually between 7 and 28 °C) is a factor influencing the growth of micro-organisms, thus ensuring favorable conditions for starter growth.[4,6,36] The influence of the processing temperature is that the higher fermentation temperature gives the starter culture the opportunity to outgrow nonstarter lactic acid bacteria.[6,36]

Initial enterobacteria counts were 2.11−2.30 Log cfu/g, with little change in quantitative terms during processing time. Similar values to those described in this experiment have been reported by other authors in dry fermented chorizo sausage[34] and salami sausage.[4]

Significant differences were not observed in the microbiota evolution in terms of the fat and konjac gel content used in the chorizo reformulation, which suggests that the processing conditions are the main factor affecting the micro-organism growth, rather than the product composition (fat levels and presence of konjac). In agreement with the results of this experiment, various authors[15,37] did not observe variations in microbial growth in fermented sausage as a function of substitution of pork backfat by an oil-in-water emulsion. No differences in lactic acid bacteria and *Enterobacteriaceae* counts were observed between the high-fat and the low-fat control fermented sausages, although in this case fat reduction was achieved through an increase in lean meat.[14,38] Similarly, no differences were observed in microbial growth from the effect of incorporating fiber into dry fermented sausages with different fat levels.[39,40]

Biogenic Amines. During the production processes (fermentation and ripening) of fermented meat products such as chorizo a series of factors concur (including the nature of the matrix and the environmental conditions), which favor growth of the micro-organisms responsible for biogenic amine formation.[1,8,36,41]

Evolution of the biogenic amine content throughout the manufacturing process of reformulated chorizo is shown in Table 2. In the raw material, at the start of the process, spermidine, agmatine, and spermine had the highest levels of biogenic amines. The presence of these amines is found naturally in meat, which is a very important source of spermine.[5,7] The concentration of these amines varies with the meat source; pork meat contains lower concentrations of

Table 1. Microbiological Counts (Log cfu/g) of Raw Material and Dry Fermented Sausage (Chorizo) Samples with Different Levels of Fat [Normal Fat (NF), Reduced Fat (RF), and Low Fat (LF)] during Fermentation and Ripening Processes[a]

		days of processing				
microorganism	sample	raw material	2	7	13	17
total viable count	NF	6.72 ± 0.12^{a1}	8.51 ± 0.23^{b1}	8.98 ± 0.42^{b1}	8.92 ± 0.06^{b1}	8.71 ± 0.05^{b1}
	RF	6.57 ± 0.15^{a1}	8.59 ± 0.05^{b1}	9.06 ± 0.03^{c1}	8.83 ± 0.02^{bc1}	8.78 ± 0.00^{bc1}
	LF	6.34 ± 0.08^{a1}	8.56 ± 0.00^{b1}	9.10 ± 0.02^{b1}	8.97 ± 0.02^{b1}	8.94 ± 0.14^{b1}
lactic acid bacteria	NF	4.65 ± 0.04^{a1}	6.48 ± 0.00^{b1}	9.09 ± 0.12^{c1}	8.95 ± 0.07^{c1}	9.00 ± 0.00^{c1}
	RF	4.54 ± 0.18^{a1}	6.48 ± 0.00^{b1}	9.18 ± 0.04^{c1}	8.82 ± 0.12^{c1}	8.91 ± 0.12^{c1}
	LF	4.69 ± 0.09^{a1}	6.48 ± 0.00^{b1}	9.18 ± 0.10^{c1}	9.06 ± 0.03^{c1}	9.05 ± 0.14^{c1}
Enterobacteriaceae	NF	2.30 ± 0.26^{a1}	2.08 ± 0.67^{a1}	2.24 ± 0.65^{a1}	2.28 ± 0.00^{a1}	2.37 ± 0.41^{a1}
	RF	2.25 ± 0.03^{a1}	1.95 ± 0.49^{a1}	2.72 ± 0.09^{b1}	3.16 ± 0.59^{c2}	2.84 ± 0.56^{bc1}
	LF	2.11 ± 0.10^{a1}	3.10 ± 1.44^{b2}	2.76 ± 0.12^{ab1}	2.13 ± 0.02^{a1}	2.30 ± 0.86^{a1}

[a]Means ± standard deviation. Different letters (a, b, c) in the same row and different numbers (1, 2, 3) in the same column indicate significant differences ($P < 0.05$).

Table 2. Biogenic Amine Contents (mg/kg) of Raw Material and Dry Fermented Sausage (Chorizo) Samples with Different Levels of Fat [Normal Fat (NF), Reduced Fat (RF), and Low Fat (LF)] during Fermentation and Ripening Processes[a]

		days of processing				
biogenic amine	sample	raw material	2	7	13	17
tyramine	NF	0.00 ± 0.00^{a1}	34.41 ± 2.65^{b1}	57.29 ± 1.83^{c1}	37.73 ± 0.25^{d1}	41.67 ± 0.12^{e1}
	RF	0.00 ± 0.00^{a1}	57.08 ± 0.10^{b2}	47.6 ± 0.18^{c2}	59.78 ± 0.21^{d2}	68.89 ± 0.15^{e2}
	LF	0.00 ± 0.00^{a1}	62.00 ± 0.20^{b3}	49.24 ± 0.19^{c3}	67.38 ± 0.16^{d3}	183.44 ± 1.20^{e3}
putrescine	NF	0.00 ± 0.00^{a1}	1.36 ± 0.26^{ab1}	6.78 ± 0.18^{b1}	17.51 ± 0.65^{c1}	18.88 ± 1.4^{c1}
	RF	0.00 ± 0.00^{a1}	7.79 ± 0.41^{b2}	31 ± 0.39^{c2}	73.87 ± 1.99^{d2}	85.59 ± 6.15^{e2}
	LF	0.00 ± 0.00^{a1}	13.12 ± 0.30^{b2}	44.84 ± 1.74^{c3}	101.99 ± 2.71^{d3}	115.92 ± 5.00^{e3}
tryptamine	NF	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.55 ± 0.29^{b1}	1.10 ± 0.30^{c1}
	RF	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	2.35 ± 0.35^{b2}	2.7 ± 0.38^{b3}
	LF	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	2.60 ± 0.16^{c2}	1.66 ± 0.10^{b2}
agmatine	NF	8.64 ± 2.40^{a1}	21.56 ± 1.40^{c2}	20.59 ± 0.14^{bc2}	21.49 ± 0.75^{c3}	18.95 ± 1.39^{b3}
	RF	7.89 ± 0.11^{a1}	26.59 ± 0.01^{e3}	21.54 ± 0.26^{d2}	15.48 ± 0.04^{c2}	13.41 ± 0.31^{b2}
	LF	12.37 ± 0.25^{b2}	17.58 ± 0.26^{c1}	6.74 ± 0.70^{a1}	6.34 ± 0.02^{a1}	6.78 ± 0.60^{a1}
spermidine	NF	1.76 ± 0.42^{b2}	1.53 ± 0.35^{ab2}	1.33 ± 0.01^{a2}	2.29 ± 0.11^{c2}	1.69 ± 0.05^{ab1}
	RF	0.61 ± 0.03^{a1}	0.82 ± 0.10^{a1}	0.95 ± 0.03^{a1}	1.82 ± 0.24^{b1}	1.46 ± 0.04^{b1}
	LF	1.41 ± 0.01^{ab2}	1.07 ± 0.15^{a1}	1.62 ± 0.18^{b2}	2.86 ± 0.16^{d3}	2.22 ± 0.09^{c2}
spermine	NF	21.47 ± 4.89^{a2}	21.92 ± 1.68^{a1}	23.25 ± 0.57^{a1}	29.33 ± 1.25^{b1}	29 ± 2.70^{b1}
	RF	12.96 ± 0.02^{a1}	21.5 ± 0.07^{b1}	25.35 ± 0.25^{b1}	36.03 ± 0.25^{c2}	40.83 ± 1.95^{d2}
	LF	19.46 ± 0.88^{a2}	20.2 ± 0.22^{ab1}	25.12 ± 1.38^{b1}	42.31 ± 0.93^{c3}	53.16 ± 2.56^{d3}

[a]Means ± standard deviation. Different letters (a, b, c) in the same row and different numbers (1, 2, 3) in the same column indicate significant differences ($P < 0.05$).

these physiological amines than beef or chicken.[24,30] Similar levels of spermidine and spermine to those observed in this study were showed by other authors in fresh dry salami and chorizo sausages[4,9,42] and frankfurter sausage-type products made with pork meat.[24]

Other biogenic amines such as histamine, phenylethylamine, cadaverine, tryptamine, tyramine, and putrescine were not detected initially. Low initial levels of these amines have also been observed by other authors in fermented meat products and related to the high quality of the ingredients, especially of the fresh meat used in the product manufacture.[4,7,36] Tyramine and putrescine along with cadaverine (in some cases) are the biogenic amines generally found in the greatest amounts in this type of product during processing.[4−6,34,43] In this study, at the end of the fermentation process (after day 2 of processing) a significant increase ($P < 0.05$) in tyramine and putrescine levels was observed depending on the KG substitution levels. The highest level of pork backfat replacement by konjac gel showed higher values ($P < 0.05$) of these biogenic amines (Table 2). In the case of putrescine this trend was maintained throughout the ripening process, as also in the case of tyramine, except on day 7 of ripening. These biogenic amines were those which presented the highest levels at the end of the storage period, mainly in the batches with KG. The increase in these biogenic amines is linked to an increased level of micro-organisms (Table 1), mainly of lactic acid bacteria and *Staphylococci*, as also reported by other authors.[4,8−12,34,43] These two main groups of bacteria are considered technologically important in the fermentation and ripening of chorizo, with proteolytic and lipolytic activity.[44] This proteolysis leads to formation of the free amino acid precursors of the biogenic amines. However, although biogenic amines are produced by decarboxylation of the free amino acids, the presence of these amino acids does not always indicate formation of the corresponding biogenic amine, since it is affected by numerous factors.[1−3] Amine formation depends on the presence of the amino acid decarboxylase enzyme of the micro-organisms and its activity in the medium.[9,11] In fact, correlations have not always been found between these FAAs and biogenic amines.[1,2] Tyrosine, histidine, ornithine, and serine seem to be better substrates for microbial metabolism.[9]

In the case of agmatine, an increase ($P < 0.05$) was also observed in the fermentation phase (Table 2), but this increment was lower in the batches which contained higher levels of konjac gel (LF), in contrast to what was observed in the case of putrescine and tyramine. A clear trend was only observed in agmatine levels as a function of the KG levels after 7 days ripening. These initial changes in the agmatine may possibly be due to formation metabolism of this biogenic amine which is formed by arginine decarboxylase activity, mainly of microbial origin. Arginine may also lead to the formation of putrescine, which in turn may lead to spermidine and spermine, as formation of these three amines is interrelated.[2] Increased levels of spermidine and spermine were also observed in a study carried out on Spanish dry-cured chorizo sausage treated with high pressure and kept in chilled storage.[43] Martuscelli et al.[45] also observed production of tyramine, spermine, and spermidine from strains of *S. xylosus* from homemade fermented sausages. Perhaps this may explain that after days 13−17 of ripening a slight decrease was observed in agmatine levels, a slight increase observed in spermidine, and a significant increase in spermine, all related to the high levels of micro-organisms detected in these batches (Table 1). Curiel et al.[10] also confirmed the presence of strains of lactic acid bacteria and

enterobacteria producing agmatine as well as those producing tyramine and putrescine associated with fresh pork sausages.

The batches which presented higher levels of spermine from day 7 onward were those reformulated with KG, with proportional increase to KG levels similar to that observed in the case of putrescine and tyramine and not so clearly in the case of spermidine. This may be due to the fact that the batches with a higher KG content have a higher humidity and protein content and lower fat content (Figure 1). It is widely recognized that humidity along with high temperatures favors microbial growth. Various studies have also shown that the fat content influences formation of biogenic amines.[4,5,46] This type of analysis has been studied more often in cheeses, observing that the concentration of biogenic amines decreases along with fat content,[47] similarly to what was observed in this study. It must also be taken into account that not only are quantitative aspects affected (microbial growth) but also growth of certain strains with greater amino acid decarboxylase capacity. Within the same species, the presence, activity, and specificity of decarboxylases are strain specific.[4,43,48] The histamine and tryptamine levels were lower than 3 mg/kg during the whole process, and phenylethylamine was not detected in any of the samples.

The levels of biogenic amines are similar to those observed in chorizo by other authors[7] and higher than those observed by others at the end of ripening[34] except in the case of putrescine, which was lower. These differences may be due to the different raw materials and processing conditions (use of starter, temperature, relative humidity, sugars, etc.), which affect the growth of different microbiota with amino acid decarboxylase capacity.[4,6,8,12,48] Mayr and Schieberle[9] also showed that different amino acid decarboxylases are active in different foods depending on the microorganisms present. However, further studies are underway to clarify the influence of processing parameters in the microorganisms and their corresponding decarboxylase enzymes responsible for formation of amines from their amino acid precursors.

Histamine levels are very low as corresponds to this type of product (with levels lower than 1 mg/kg) and were only observed in the final days of ripening. Similar behavior was observed for tryptamine, where levels were only detected on days 13 and 17 of ripening, with higher levels (2.35−2.7 mg/kg) in the reformulated batches compared with the control batch. The cadaverine levels throughout ripening were <1 mg/kg.

In relation to the safety of these compounds it should be taken into account that consumption of foodstuffs with high levels of the tyramine (vasoactive substance) has been related to different toxic processes, reported as migraine, headache, and raised blood pressure.[1,3] Besides being toxic in itself, recent studies on tyramine have shown that it promotes adhesion to the gastric mucosa by pathogens like *Escherichia coli* O157:H7.[49] It should also be considered that the presence of putrescine enhances the toxicity of tyramine and is therefore also implicated in these processes.[1] However, this toxicity also depends on the detoxification system of the consumer. The human body can rapidly detoxify the histamine and tyramine absorbed from foods by means of the enzymes monoamine oxidase (MAO; EC 1.4.3.4), diamine oxidase (DAO; EC 1.4.3.6), and polyamine oxidase (PAO; EC 1.5.3.11), which play a major role in amine degradation in the human body.[1−3] However, these detoxification mechanisms may be altered by genetic factors, if large amounts are ingested, or if the individual consumer is undergoing treatment with oxidase enzymes (e.g., monoamine oxidase inhibitor, MAOI), which inhibit amino-oxidases or cause amino-oxidase deficiency.[2,3,44] Determination of the exact toxicity threshold of biogenic amines in individuals is extremely difficult. The toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of different individuals. The amount of tyramine considered toxic ranged from 125 to 800 mg/kg.[2] In contrast, 6 mg/kg of tyramine would be toxic if ingested with MAOIs.[1,2] This is particularly important at the present day because of the high consumption of MAOIs as antidepressants. Studies of biogenic amine concentrations in commercially processed Spanish meat products reported that 63% of salchichon sausage samples and 64% of chorizo sausage samples (both ripened meat products) contained enough tyramine to poison consumers taking MAOIs.[50] Other amines like spermidine and spermine have also been associated with food allergies.[3] In this study, these products reformulated with KG seem unlikely to pose any health risk to consumers due to the level of amines present in them.

In this context, the reformulation process of reduced- and low-fat dry fermented sausages with partial replacement of pork backfat by konjac gel modifies the biogenic amine profile without affecting microbial development to any relevant extent and without compromising product safety. Fat reduction linked to the presence of konjac gel favors production of certain biogenic amines (tyramine, putrescine, and spermine) and reduces production of agmatine in dry fermented sausage. Design of healthier meat products, reducing fat content and promoting inclusion of konjac gel, is a promising avenue of research, especially as the safety of these products in relation to the presence of biogenic amines is guaranteed.

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IV.2.3. Healthy oil combination stabilized in a konjac matrix as pork fat replacement in low-fat, PUFA-enriched, dry fermented sausages.

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Healthy oil combination stabilized in a konjac matrix as pork fat replacement in low-fat, PUFA-enriched, dry fermented sausages

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ABSTRACT

Dry fermented sausage ("Chorizo") was reformulated to produce better lipid compositions (reduced fat content and improved fatty acid profile) by replacing the pork backfat by a healthier oil combination (olive, linseed and fish oils) stabilized in a konjac matrix. Proximate analysis, fatty acid profiles, lipid oxidation and sensory analysis were studied. The fat content was 99–130 g/kg in the low-fat versus 316 g/kg in the normal fat sausages. The incorporation of an oil-in-konjac matrix reduced ($P < 0.05$) saturated fatty acid content and increased ($P < 0.05$) polyunsaturated fatty acids (PUFA), improving the $n-6/n-3$ ratio in dry sausage. Reducing fat decreased ($P < 0.05$) hardness and increased ($P < 0.05$) cohesiveness, with no effect ($P > 0.05$) on springiness and chewiness. The reformulation process produced a decrease ($P < 0.05$) in all sensorial parameters, compared with the control sample, although in all the sausages appearance, flavour and juiciness scored above the middle value on the hedonic scale.

1. Introduction

Dry fermented sausages such as "chorizo" in Spain are popular traditional meat products in various countries, although they present some negative health concerns related to their high fat (250–450 g/kg), energy content (1256–1885 kJ/100 g) and the fatty acid profiles of animal fat (Muguerza, Gimeno, Ansorena, & Astiasarán, 2004). This has led the meat industry to modify traditional formulations to make them healthier. As in other meat products with similar characteristics, dry fermented sausage reformulation processes have been used to reduce fat content and/or to improve the fatty acid profile (Jiménez-Colmenero, 2007; Muguerza et al., 2004; Ruiz-Capillas, Triki, Herrero, Rodriguez-Salas, & Jiménez-Colmenero, 2012).

Different studies have explored possible fat reduction in dry fermented sausages, using partial fat replacement by lean meat as a formulation strategy (Liaros, Katsanidis, & Bloukas, 2009; Muguerza, Fista, Ansorena, Astiasarán, & Bloukas, 2002; Olivares, Navarro, Salvador, & Flores, 2010). In some cases this strategy has been accompanied by the addition of other ingredients such as inulin (Mendoza, García, Casas, & Selgas, 2001), cereal and fruit fibres (García, Dominguez, Galvez, Casas, & Selgas, 2002) and short-chain fructooligosaccharides (Salazar, García, & Selgas, 2009) to obtain a low calorie content and contribute to ensuring the desired product characteristics. However, these reformulation processes often increase the product toughness due to higher water loss during fermentation (Muguerza et al., 2002; Olivares et al., 2010; Salazar et al., 2009). Visual differences in the product appearance also occur as there is less granulated fat as the fat content is reduced. In dry fermented sausage, granulated fat has a further technological function as it is involved in moisture release, a necessary process for undisturbed fermentation and flavour/aroma development (Muguerza et al., 2002). In this context, the use of konjac gel as a fat analogue opens up interesting possibilities for reducing fat in this type of meat products (Ruiz-Capillas et al., 2012). Konjac glucomannan (KGM) is a neutral polysaccharide produced by the *Amorphophallus konjac*, a native plant of East Asia, where it has been used since ancient times. Konjac flour is considered a low calorie ingredient (E-425) which, given its content in non-digestible fibre, presents numerous physiological effects and therapeutic applications (Ruiz-Capillas et al., 2012). When KGM is dissolved in an alkaline coagulant (such as calcium hydroxide), deacetylation occurs and a thermally stable gel is formed (Lin & Huang, 2003) Konjac gel, when ground down to a desired particle size can achieve the appearance (as visible granulated fat) required for use as raw material to replace animal fats. The authors (Ruiz-Capillas et al., 2012) studied the effect of animal fat replacement (fractions up to 0.80 of pork backfat) by an equal proportion of

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konjac gel on processing and quality characteristics of reduced and low-fat fermented dry sausage. Although the products obtained presented acceptable sensory characteristics, high levels of animal fat replacement by konjac gel may present undesirable results in weight loss, hardness and juiciness, which must be limited to boost the use of this technology. In order to limit these undesirable results when improving processing and quality characteristics of dry fermented sausage, it would be advisable to modify konjac gel properties used as fat analogue to improve its water-binding properties (to hinder evaporation in the drying process) thus reducing weight loss and hardness and improving juiciness (Ruiz-Capillas et al., 2012).

Studies have also been carried out to improve the fat content of meat products, including both reducing fat level and improving fatty acid profile. It should be noted here that both types of strategies are not normally linked due to the difficulties in terms of the manufacturing technology and products physicochemical characteristics involved. Many studies have reported new formulations for healthy fermented sausages using vegetable and marine oils (olive, soy, linseed, canola, fish, etc.) as partial substitutes for animal fat (Muguerza et al., 2004; Muguerza, Gimeno, Ansorena, Bloukas, & Astiasaran, 2001; Pelser, Linssen, Legger, & Houben, 2007; Valencia, Ansorena, & Astiasaran, 2006). The addition of individual lipids (of plant or marine origin) improves the fatty acid profile of meat products, but a better approximation to an optimal lipid profile from a health standpoint can be achieved using healthier oil combinations as animal fat replacers (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2010). With this aim, combinations of linseed and algae oils have been used in the formulation of healthier dry fermented sausage (García-Iníguez de Ciriano et al., 2010). In previous papers, our research group assessed the suitability of a healthier oil combination as pork backfat replacement in meat products, such as frankfurters (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2010) and paté (Delgado-Pando, Cofrades, Rodriguez-Salas, & Jiménez-Colmenero, 2011). The healthier lipid combination was formed by vegetable oils (olive and linseed) and fish oils in suitable amounts and proportions to provide a fatty acid profile better adjusted to healthier intake goals. This oil combination was designed to produce a lipid material with a small proportion of SFA, large proportions of MUFA and PUFA and balanced $n-6/n-3$ PUFA and PUFA/SFA ratios (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010).

Plant and marine oils have been incorporated into fermented sausage in liquid, solid or encapsulated forms, but the most widely assayed method is stabilized oil-in-water emulsion (Jiménez-Colmenero, 2007). The development of a technology incorporating healthy oils into a konjac matrix and its use as a strategy for improving the fat content of meat products represents a new proposal for oil stabilization, with additional health benefits linked to the presence of dietary fibre (Lin & Huang, 2003). It also offers the possibility of enhancing the properties of the konjac gels needed to improve their suitability as fat analogues in dry fermented sausage (Ruiz-Capillas et al., 2012). As far as the authors are aware, the use of a healthier oil combination stabilized into a konjac matrix as a functional ingredient and animal fat replacement in the development of low-fat and PUFA enriched dry fermented sausage has not been explored.

The aim of this paper is to evaluate the nutritional consequences and quality characteristics of a dry fermented sausage reformulation process to produce better lipid compositions (reducing fat content and improving the fatty acid profile) by replacing pork backfat by a healthier oil combination (olive, linseed and fish oils) stabilized in a konjac matrix. Proximate analysis, fatty acid profiles, texture and sensory analysis of low-fat and PUFA-enriched dry fermented sausages. Studies on shelf life of this products will be reported in another paper.

2. Materials and methods

2.1. Meat raw material, ingredients and additives

Fresh post-rigor pork meat (mixture of *M. biceps femoris*, *M. semimembranosus*, *M. semitendinosus*, *M. gracilis* and *M. adductor*) (224, 50, 728 g/kg of protein, fat and moisture contents, respectively) and pork backfat (70, 885 and 32 g/kg of protein, fat, and moisture contents, respectively) were obtained from a local market and frozen at −20 °C until used (not more than 14 days later).

Konjac materials used as animal fat replacement were made with konjac flour (glucomannan 830 g/kg, 120 mesh) from Trades S.A. (Barcelona, Spain), pre-gelled cornstarch (Amigel, Julio Criado, S.L. Madrid. Spain). i-carrageenan (Hispanagar S.A, Burgos, Spain) and $Ca(OH)_2$ (Panreac Química S.A., Barcelona, Spain). Olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain), linseed oil (Natursoy S.L., Alimentos Ecológicos, Castellterçol, Spain) and fish oil (Omevital 18/12 TG Gold, Cognis GMBH, Illertissen, Germany), the latter containing according to the producer 160 mg eicosapentaenoic acid (EPA)/g and 115 mg docosahexaenoic acid (DHA)/g, were used to prepare the healthier oil combination to be incorporated into the konjac matrix. This oil combination was prepared with 443.9 g/kg olive oil, 378.7 g/kg linseed oil and 177.4 g/kg fish oil (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010). Other ingredients and additives used were sodium chloride (Panreac Química, S.A. Barcelona, Spain), sodium tripolyphosphate (STP) (Manuel Riesgo, S.A. Madrid, Spain), sodium nitrite (Fulka Chemie GMBH, Buchs, Germany), and two commercial prepared "choravi" (color/flavour salts) and "curavi" (curing salts) from ANVISA (Arganda del Rey, Spain).

2.2. Konjac material preparation

Three types of konjac materials were prepared for use in dry fermented sausage formulation: one with no added oil (KG) and the other two incorporating different oil combination fractions (0.10 and 0.20) (K10 and K20, respectively) into a konjac matrix (oil-in-konjac matrix). The different types of konjac materials were prepared as follows: The KG preparation was based on that of Osburn and Keeton (2004) with modifications (Jiménez-Colmenero et al., 2010). Briefly, konjac flour (50 g/kg) was homogenized (Stephan Universal Machine UM5, Stephan Machinery GmbH and Co., Hameln, Germany) with 648 g/kg of added water for 180 s, left to rest for 300 s then homogenized for a further 180 s; i-carrageenan (10 g/kg) was then added and the mixture homogenized again for 180 s. Pre-gelled cornstarch powder (30 g/kg) was dispersed in 162 g/kg of added water and homogenized with the konjac flour and i-carrageenan mixture, left to rest for 300 s then homogenized for a further 180 s. The mixture was cooled to 10 °C, then 10 g/kg of a $Ca(OH)_2$ solution (0.01) was added with gentle stirring at room temperature.

The oil-in-konjac matrix (K10 and K20) was prepared in the same way as KG, although in every case, the corresponding oil combination amount was added just after the addition of the i-carrageenan, and the mixture homogenized for 180 s. The three types of konjac materials (KG, K10 and K20) were formulated maintaining, on aqueous basis (without considering the added oil), the same proportions of components used in its preparation. The technological viability of the incorporation of the oils into konjac materials was established previously in our laboratory.

The three types of konjac materials were prepared in duplicate and placed in suitable containers, covered, manually overpressured to eliminate air and stored at 2 ± 2 °C until used (within 24 h of preparation).

2.3. Design and production of dry fermented sausage

Dry fermented sausages were designed and formulated to reduce fat content and/or to improve the fatty acid profile, using a similar amount of lean meat (and therefore basically of muscle protein), since the fat reduction was achieved by replacing pork backfat by the same proportion of konjac materials. Four different formulations of dry fermented sausage ("chorizo") were elaborated (Table 1): a control sample, prepared with normal fat content (NF), a low fat sample (LFKG) reformulated replacing pork backfat by KG, and finally two low fat samples (LFK10 and LFK20) reformulated replacing pork backfat by the same proportion of K10 and K20, respectively.

The meat and backfat were thawed before use (18 h at 2 ± 2 °C). The sausages were manufactured as follows: First, the meat, backfat and konjac materials were minced at 15 mm (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy). The backfat and/or konjac materials (depending on the dry sausage sample formulation) were thoroughly mixed manually with the meat and then minced again at 15 mm. This batter was homogenized for 60 s (MAINCA, Granollers, Barcelona, Spain), then half of the additives (choravi, curavi and NaCl), were added and the mixture homogenized again for 60 s. The remaining additives were then added and the mixture homogenized for 120 s more. In all cases the final temperature was less than 11 °C. Immediately, the prepared sausage mixture was manually stuffed using a stuffer (MAINCA, Granollers, Barcelona, Spain) into 4 cm diameter collagen casing (Fibran S. A. Sant Joan de les Abadesses, Gerona, Spain). The sausages were hand-linked to standard sizes (22–23 cm), which were placed in a ripening cabinet (BINDER model KBF 240 Tuttlingen, Germany) programmed to operate under the following conditions: 48 h at 23 °C, 90% relative humidity (RH), then at 13 °C, 70–80% RH. The end of the ripening process was defined when weight loss (in relation to the initial weight) of the sausages reached 370–420 g/kg. The dry fermented sausages were packed in plastic bags (BB3050 Cryovac® Spain) (under aerobic conditions) until analysis.

2.4. Proximate composition and energy content

Sample moisture and ash contents were determined (AOAC, 2005) in triplicate in all samples. Protein content was measured in quadruplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). Fat content was evaluated in triplicate according to Bligh and Dyer (1959). Carbohydrates were calculated taking into account the ingredient composition and formulation content, as well as the water loss during ripening. Energy value was estimated from protein (×17.15 kJ/g), carbohydrate (×17.15 kJ/g) and fat (×38.07 kJ/g) contents for each product.

Table 1
Formulation (g/kg) of dry fermented sausages.[a]

Samples	Meat	Backfat	Fat replacer		
			Konjac gel	Oil-in-konjac matrix	
				K10	K20
NF	739	185	–	–	–
LFKG	739	46.2	138	–	–
LFK10	739	22.0	–	163	–
LFK20	739	–	–	–	185

[a] Samples: NF, dry fermented normal fat sausage formulated with all pork fat; LFKG, dry fermented low-fat sausage reformulated replacing pork backfat by konjac gel; LFK10 and LFK20, dry fermented low-fat sausage reformulated replacing pork backfat by an oil-in-konjac matrix (K10 and K20) containing 100 and 200 g/kg of healthy oil combination (0.44 olive oil, 0.38 linseed oil and 0.18 fish oil fractions), respectively. The following were also added to all samples: 55 g/kg of "choravi", 2.7 g/kg of "curavi" and 18.5 g/kg sodium chloride.

2.5. Fatty acid profile

The fatty acid composition of the sausages was determined (in sextuplicate) by gas chromatography and the fatty acids were identified by comparison with a known standard FAME mixture (Supelco, Alltech Associated, Inc. Deerfield, IL, USA) and quantified as reported by Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al. (2010).

2.6. Texture profile analysis (TPA)

TPA, as described by Bourne (1978), was carried out using a TA.XT2i Stable Micro Systems Texture Analyser (Stable Micro Systems Ltd., Surrey, England). Ten cores (diam = 20 mm, height = 20 mm) per sample were axially compressed to 50% of their original height. A 30 kg load cell was used at a crosshead speed of 1 mm/s. Attributes were calculated as follows: hardness (Hd) = peak force (N) required for first compression; cohesiveness (Ch) = ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp) = distance (mm) the sample recovers after the first compression; chewiness (Cw) = Hd × Ch × Sp (N × mm).

2.7. Sensory analysis

Sensory evaluation of each product was performed by 16 panellists previously trained with various training sessions in the products and terminology. For this purpose commercial dry fermented sausages were used. The samples were presented to the panellists in 2 mm thick oblique slices. A hedonic scale rating test was carried out where testers evaluated the following for each sample: appearance (0 = not characteristic, 10 = characteristic), flavour (0 = very mild, 10 = very strong), firmness (0 = very soft, 10 = very hard), juiciness (0 = very dry, 10 = very juicy), and overall acceptability (0 = dislike extremely, 10 = like extremely).

2.8. Statistical analysis

A one-way analysis of variance (ANOVA) was carried out to evaluate the statistical significance ($P < 0.05$) in the characteristics of the different fermented sausages. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, Ill., U.S.A.). The experiment was replicated twice.

3. Results and discussion

3.1. Proximate composition and energy content

According to the experimental design, the proximate composition varied ($P < 0.05$) with product formulation (Table 2). The fat content of the normal fat sausage (NF) was 316 g/kg, similar to that of conventional sausage (García-Iníguez de Ciriano et al., 2010; Mendoza et al., 2001). The fat content of the low fat sausages (LFKG, LFK10 and LFK20) ranged from 99 to 130 g/kg, showing an important fat reduction (0.58–0.68) as compared with the NF sample. Fat reduction was accompanied by an increase ($P < 0.05$) in moisture and carbohydrate content, similar ($P > 0.05$) ash proportion, but lower ($P < 0.05$) protein level in the case of samples with added oils

Table 2
Proximate analysis and energy content in dry fermented sausages.

	Samples			
	NF	LFKG	LFK10	LFK20
Moisture (g/kg)	317.0 ± 22.7[a]	475.8 ± 27.8[b]	505.9 ± 16.4[b]	476.0 ± 17.4[b]
Fat (g/kg)	316.0 ± 10.5[a]	129.9 ± 4.3[b]	105.7 ± 3.5[c]	98.8 ± 3.3[c]
Protein (g/kg)	295.5 ± 9.8[a]	290.5 ± 8.9[a]	255.0 ± 8.5[b]	268.1 ± 9.7[b]
Ash (g/kg)	66.5 ± 1.0[b]	60.1 ± 0.8[a]	61.4 ± 1.1[a]	69.0 ± 0.5[b]
Carbohydrates (g/kg)	39.2 ± 1.3[a]	61.0 ± 3.9[b]	64.2 ± 2.1[bc]	67.0 ± 2.2[c]
Fat reduction (fraction of NF)	–	0.58	0.66	0.68
Energy value (kJ/100 g)	1778.6	1083.5	950.4	951.6
Fraction of energy reduction of NF	–	0.39	0.46	0.46
kJ (fraction from fat)	0.68	0.46	0.42	0.39
kJ (fraction from pork fat)	0.68	0.46	0.33	0.20
kJ (fraction from oils)	0	0	0.07	0.15
kJ (fraction from SFA)	0.24	0.17	0.13	0.09
kJ (fraction from MUFA)	0.30	0.20	0.18	0.18
kJ (fraction from PUFA)	0.08	0.06	0.09	0.10

Samples: NF, dry fermented normal fat sausage formulated with all pork fat; LFKG, dry fermented low-fat sausage reformulated replacing pork backfat by konjac gel; LFK10 and LFK20, dry fermented low-fat sausage reformulated replacing pork backfat by an oil-in-konjac matrix containing 100 and 200 g/kg of healthy oil combination (0.44 olive oil, 0.38 linseed oil and 0.18 fish oil fractions), respectively.
Means ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

(LFK10 and LFK20). These results can be attributed basically to the differences in product formulation (Table 1) and, according to experimental conditions (see M&M), to a lesser extent to differences in weight loss occurring in the different formulations during ripening. Different fat reduction levels in dry fermented sausages (in some case nearly 0.60) have been reported (García et al., 2002; Mendoza et al., 2001; Muguerza et al., 2002; Salazar et al., 2009). Fat reduction of up to 0.54 has been reported in dry fermented sausages where pork backfat replacement by konjac gel is used as a fat reduction strategy (Ruiz-Capillas et al., 2012). However, the fat reduction process was not accompanied in any of the above cases by modifications of the lipid fraction using vegetable and marine oils as partial substitutes for animal fat (Jiménez-Colmenero, 2007).

In terms of ingredient composition and formulation, around a fraction 0.16 and 0.37 (1.66 and 3.70 g oil/100 g sausage) of fats contained in LFK10 and LFK20 respectively, were supplied by the oils incorporated into the konjac material. This means that the dry fermented sausages contained 0.73–1.62 g, 0.62–1.40 g and 0.29–0.65 g (per 100 g of the product) of olive, linseed and fish oils respectively.

Energy values for the dry fermented sausage ranged from 1778.6 kJ/100 g for NF sample to 950–1084 kJ/100 g (Table 2) for low fat sausages. The fat contribution to the energy value was lower in low-fat samples, with a fraction of 0.68 of the total caloric value fat-derived in NF, and around 0.40 in low-fat samples. Additionally, it should also be taken into account that as the amount of pork backfat was replaced by oil-in-konjac matrix, the calorie content from animal fat was also reduced in favour of the contribution from the oil combination (Table 2). Different fractions of total energy reduction (up to 0.35) have been reported as a result of fat reduction in dry fermented sausage (García et al., 2002; Mendoza et al., 2001; Salazar et al., 2009). Energy reduction of up to 0.24, has been reported in dry fermented sausages using pork backfat replacement by konjac gel as a fat reduction strategy (Ruiz-Capillas et al., 2012).

3.2. Fatty acid profile

The fatty acid composition of dry sausages (Table 3) was clearly different in samples made with all-pork fat (NF and LFKG) and those produced with oil-in-konjac matrix (LFK10 and LFK20). The most abundant fatty acids in the all-pork fat dry sausages (NF and LFKG) were MUFA, followed by SFA and PUFA; MUFA and PUFA together accounted for around 600 g/kg of total fatty acids. In these samples, oleic acid was the most abundant fatty acid, followed by palmitic, stearic and linoleic acids. These results are consistent with reports of the fatty acid composition of products formulated with pork fat (Ayo et al., 2007; Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2010). Differences in fatty acid profile between NF and LFKG are possibly due to the different sources of the pork fats used in their formulation (Ayo et al., 2007). According to formulation conditions the proportion of intramuscular fat was higher in LFKG sausage while the proportion of depot fat (backfat) was higher in the NF sample. Intramuscular fat contained lower proportions of SFA and MUFA and higher proportions of PUFA than removable depot fat. Leaner cuts containing a higher percentage of phospholipids had higher percentages of PUFA (Raes, De Smet, & Demeyer, 2004).

Table 3
Fatty acid profile (as g/kg of total fatty acids) and nutritional significant ratios of dry fermented sausages.

Fatty acid	Dry fermented sausages			
	NF	LFKG	LFK10	LFK20
Myristic C14:0	12.3 ± 0.2[b]	13.1 ± 0.9[b]	12.7 ± 0.9[b]	9.1 ± 0.1[a]
Palmitic C16:0	230.8 ± 2.6[c]	233.9 ± 2.6[c]	197.5 ± 3.5[b]	157.1 ± 2.8[a]
Stearic C18:0	109.7 ± 0.6[c]	128.4 ± 11.0[d]	101.2 ± 1.8[b]	72.8 ± 1.8[a]
∑SFA	375.1 ± 21.9[c]	384.2 ± 20.1[c]	331.6 ± 6.8[b]	248.6 ± 3.5[a]
Palmitoleic C16:1	21.3 ± 0.3[b]	19.0 ± 0.6[a]	22.0 ± 0.2[c]	18.8 ± 0.8[a]
Oleic C18:1n9	404.7 ± 19.1[a]	397.8 ± 15.3[a]	383.0 ± 4.9[a]	389.4 ± 2.4[a]
Vaccenic C18:1n7c	38.1 ± 0.2[d]	34.9 ± 1.0[c]	32.2 ± 0.6[b]	30.2 ± 0.6[a]
Eicosenoic C20:1n9c	13.5 ± 0.2[d]	11.3 ± 0.6[c]	7.9 ± 0.2[b]	5.8 ± 0.0[a]
∑MUFA	474.1 ± 24.1[ab]	465.7 ± 15.4[ab]	446.4 ± 3.7[a]	475.0 ± 3.6[b]
Linoleic C18:2n6	101.5 ± 0.7[a]	125.1 ± 4.7[b]	123.1 ± 1.4[b]	137.9 ± 1.5[c]
Linolenic C18:3n3	6.3 ± 0.1[a]	5.9 ± 0.4[a]	82.0 ± 1.0[b]	101.2 ± 1.1[c]
Eicosapentaenoic C20:5n3	–	–	9.2 ± 0.2[a]	10.8 ± 0.3[b]
Docosahexaenoic C22:6n3	–	–	5.8 ± 0.1[a]	7.5 ± 0.0[b]
∑PUFA	124.1 ± 0.7[a]	145.6 ± 3.9[b]	227.4 ± 1.9[c]	254.3 ± 6.9[d]
PUFA/SFA	0.33 ± 0.01	0.37 ± 0.03	0.76 ± 0.02	1.02 ± 0.04
∑*n*−3	10.1 ± 0.1[a]	12.8 ± 0.5[b]	104.1 ± 1.1[c]	119.5 ± 8.5[d]
∑*n*−6	114.0 ± 0.7[a]	132.8 ± 3.4[c]	124.2 ± 1.8[b]	137.9 ± 1.5[d]
n−6/*n*−3	11.25 ± 0.15	10.39 ± 0.13	1.19 ± 0.03	1.19 ± 0.10

Samples: NF, dry fermented normal fat sausage formulated with all pork fat; LFKG, dry fermented low-fat sausage reformulated replacing pork backfat by konjac gel; LFK10 and LFK20, dry fermented low-fat sausage reformulated replacing pork backfat by an oil-in-konjac matrix containing 100 and 200 g/kg of healthy oil combination (0.44 olive oil, 0.38 linseed oil and 0.18 fish oil fractions), respectively.
Means ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

The incorporation of the oil-in-konjac matrix produced major changes in the fatty acid profiles of dry sausages (Table 3). As compared with the all-pork fat samples, these reformulated products contained less ($P < 0.05$) SFA, including palmitic acid. The concentrations of palmitic and myristic acids decreased from around 245 g/kg (NF and FLKG) to 170 g/kg of total fatty acids in LFK20 sample. No significant variations were observed in MUFA content between the all-pork fat samples (NF and LFKG) and those containing non-animal fat (LFK10 and LFK20), while the oleic content was around 390–400 g/kg of total fatty acids in both types of products (Table 3). Pork fat replacement by oil combination increased ($P < 0.05$) the linolenic (ALA), EPA, and DHA fatty acids content in dry sausages. Based on the fatty acid and fat contents of the products total $n-3$ PUFA were around 1.12 g/100 g (of which approximately 0.95 g/100 g was ALA and 170 mg/100 g were long chain $n-3$ PUFA) in LFK20 sample as opposed to around 0.303 3.03 and 0.157 g/100 g in NF and LFKG, respectively (all-pork fat products). Many studies have reported healthier lipid formulations for fermented sausages using vegetable and marine oils as partial substitutes for animal fat; however these products prepared with different types of oils (olive, soy, linseed, canola, fish, algae, etc.) contain high fat concentrations, generally over 250 g/kg (García-Iníguez de Ciriano et al., 2010; Jiménez-Colmenero, 2007; Valencia, Ansorena, & Astiasaran, 2007). For this reason the $n-3$ PUFA enrichment produced in LFK20 sausage is of greater relevance as it is accompanied by important reduction of fat and energy fractions of around 0.70 and 0.46 respectively (Table 2), and it is also linked to a very notable decrease in the $n-6/n-3$ ratio (11.25 versus 1.19) (Table 3), as compared with the normal fat product (NF). In terms of energy contribution, the incorporation of oil-in-konjac matrix in dry sausage reduces the proportion of calories from SFA and MUFA and increases those provided by PUFA (Table 2).

The changes in the fatty acid profile of dry sausage as a result of pork fat replacement by oil combination are consistent with the fact that the fatty acid composition of oil stabilized in konjac is characterized by a low proportion of SFA, and a high proportion of MUFA (mainly oleic acid, in similar levels of pork fat) and $n-3$ PUFA (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2010). Variations in similar fatty acid profiles have been described by Delgado-Pando, Cofrades, Ruiz-Capillas, and Jiménez-Colmenero (2010) using oil (healthier lipid combination)-in-water emulsions as pork backfat replacers in low-fat frankfurters.

3.3. Texture profile analysis

TPA parameters were affected ($P < 0.05$) by formulation (Fig. 1). Reducing fat by partial replacement of animal fat with konjac gel (NF versus LFKG) decreased ($P < 0.05$) hardness and increased ($P < 0.05$) cohesiveness, with no effect ($P > 0.05$) on springiness and chewiness. In contrast to these findings, the formation of harder structures has been reported as fat content decreases in dry fermented sausages (Liaros et al., 2009; Muguerza et al., 2002, 2004). Ruiz-Capillas et al. (2012) also reported that as fat content decreases (and konjac gel levels increase) there is an increase in hardness and chewiness, while cohesiveness is reduced. This apparent discrepancy may be attributed to the different characteristics of the products obtained as the result of the different criteria used to establish the completion of the ripening process in both experiments. Since in this experiment the end of the ripening process was defined by the level of weight loss (and not by ripening time), the moisture levels of the low-fat dry fermented sausages were higher than those reported by Ruiz-Capillas et al. (2012), resulting in products with a higher moisture/protein ratio which would explain the lower hardness of these products.

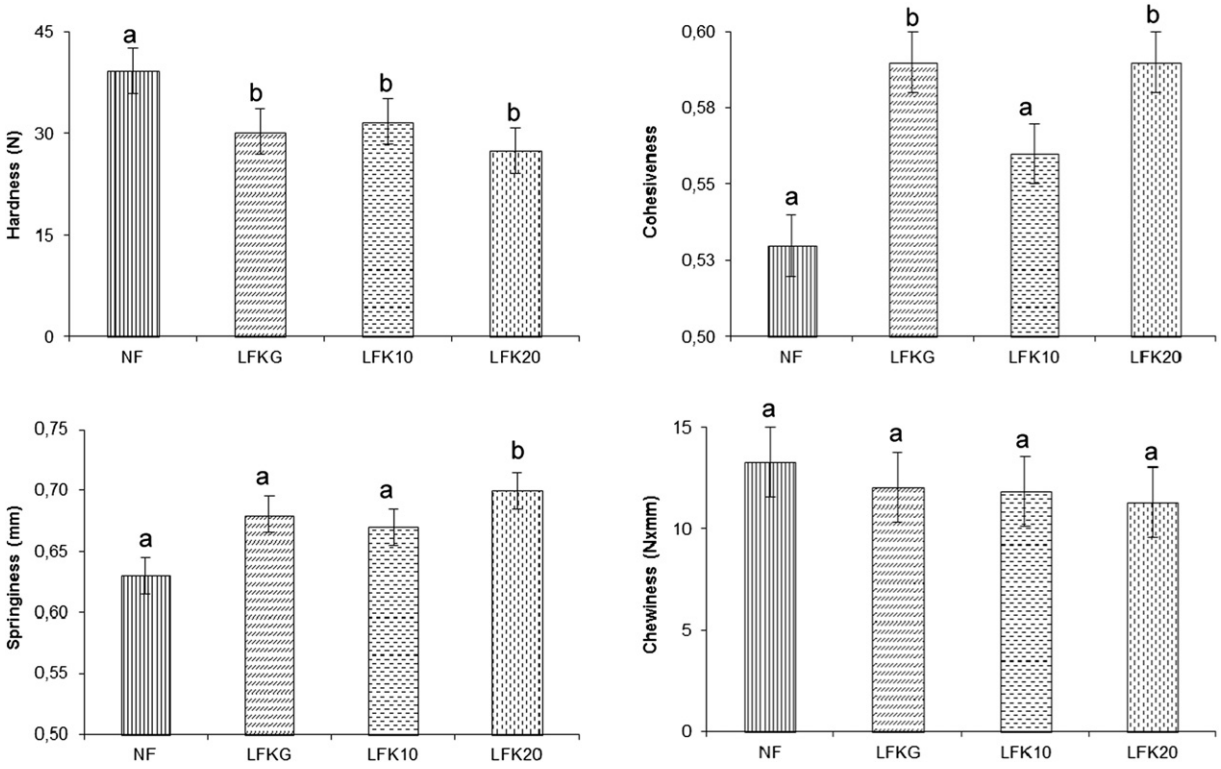

Fig. 1. TPA parameters of dry fermented sausage.

Table 4
Sensory analysis of dry fermented sausages.

Samples	Appearance	Flavour	Firmness	Juiciness	Acceptability
NF	8.54 ± 0.66^{a}	7.70 ± 0.40^{a}	6.06 ± 0.93^{a}	7.71 ± 0.66^{a}	7.70 ± 0.87^{a}
LFKG	6.74 ± 1.18^{b}	6.73 ± 0.80^{b}	5.09 ± 1.09^{b}	4.83 ± 1.08^{b}	6.39 ± 0.99^{b}
LFK10	6.53 ± 0.76^{b}	5.61 ± 1.03^{c}	3.18 ± 0.85^{c}	5.01 ± 0.98^{b}	4.87 ± 0.84^{c}
LFK20	6.19 ± 1.33^{b}	6.17 ± 0.53^{c}	2.77 ± 0.92^{c}	5.19 ± 1.02^{b}	2.76 ± 0.41^{d}

Samples: NF, dry fermented normal fat sausage formulated with all pork fat; LFKG, dry fermented low-fat sausage reformulated replacing pork backfat by konjac gel; LFK10 and LFK20, dry fermented low-fat sausage reformulated replacing pork backfat by an oil-in-konjac matrix containing 100 and 200 g/kg of healthy oil combination (0.44 olive oil, 0.38 linseed oil and 0.18 fish oil fractions), respectively. Means ± standard deviation. Different letters in the same column indicate significant differences ($P < 0.05$).

3.4. Sensory evaluation

Table 4 shows the sensory scores for dry fermented sausages. The reformulation process produced a decrease ($P < 0.05$) in all sensorial parameters, compared with the control sample (NF), although in all the sausages appearance, flavour and juiciness scored above the middle value on the hedonic scale. These changes seem to be mainly attributable to the fat reduction. Although the visual aspect of the konjac material included in the meat matrix is similar to the fat it replaces, as reported by other authors (Liaros et al., 2009; Muguerza et al., 2002), fat reduction also decreases the appearance of dry fermented sausage. No differences were found in appearance and flavour of dry sausages affected by fat levels and konjac gel addition, in this case without oils incorporated in the konjac matrix (Ruiz-Capillas et al., 2012). Since the variation in the juiciness score of dry sausage does not seem to be linked to moisture content (Table 2), these differences may be conditioned by the different levels of water released by chewing. The better water-binding properties of konjac materials as compared with muscle structure (together with a lower fat level) would produce the release of less fluids during chewing, resulting in reduced juiciness (Ruiz-Capillas et al., 2012).

The greatest sensorial limitation induced by reformulation is in firmness (Table 4), the parameter which can be considered as mainly responsible for the decrease in the general acceptability of products elaborated with oil-in-konjac matrix. Both fat reduction and the presence of oil-in-konjac matrix produced softer structures in meat products, decreasing ($P < 0.05$) the firmess score (Table 4). Overall, the panel considered that the sensory characteristics of the NF, LFKG and LFK10 products were scored in the upper part of the scale.

4. Conclusion

In this study a new reformulation procedure has been applied to improve the fat content of dry fermented sausage, both by reducing the fat proportion and improving the fatty acid profile. To do this, an unexplored technological option was adopted based on replacing pork backfat by konjac gel and a healthier oil combination (olive, linseed and fish oils) stabilized in a konjac matrix. This is an appropriate strategy to improve the product fat content since it enables an important fat and energy value reduction as compared with normal fat products accompanied by $n-3$ PUFA enrichment with an associated very marked decrease in the $n-6/n-3$ ratio. The results of this study suggest that further modifications should be considered in product formulations to improve texture and sensorial attributes.

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IV.2.4. Chilled storage characteristics of low-fat, n-3 PUFA-enriched dry fermented sausage reformulated with a healthy oil combination stabilized in a konjac matrix.

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Chilled storage characteristics of low-fat, n-3 PUFA-enriched dry fermented sausage reformulated with a healthy oil combination stabilized in a konjac matrix

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ABSTRACT

A healthier oil combination (olive, linseed and fish oils) stabilized in a konjac matrix was used as pork fat replacement to reformulate low-fat, n-3 polyunsaturated fatty acid (PUFA) enriched dry fermented sausage ("chorizo"). Different characteristics were studied during chilled storage: pH, weight loss, water activity, texture profile, lipid oxidation, microbiological changes and biogenic amines formation. The pH values of the dry sausage were not affected ($P > 0.05$) by the sausage formulation and storage period and a minor effect on weight loss was observed. The water activity (a_w) values ranged from 0.856 to 0.896. Hardness increased ($P < 0.05$) during chilling storage. Lipid oxidation increased ($P < 0.05$) with storage time, more noticeably in samples with higher PUFA content. No limitations related to microbiological aspects and the formation of biogenic amines were found as an effect of the formulation strategy and chilled storage. Reformulation affected the presence of tyramine and cadaverine. The biogenic amines concentration generally increased ($P < 0.05$) with storage time (mainly in tyramine, histamine, putrescine and cadaverine), although the increase varied with formulation and storage period.

1. Introduction

Healthier lipid formulation based on processing strategies is currently one of the most important approaches to the development of potential meat-based functional foods (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2010; Jiménez-Colmenero, 2007). This is specially relevant in dry fermented sausages such as "chorizo", a very popular product in Spain and other countries which generally presents high fat levels (25–45%) and energy content (300–450 kcal/100 g) (Jiménez-Colmenero, Triki, Herrero, Rodriguez-Salas, & Ruiz-Capillas, in press; Muguerza, Gimeno, Ansorena, & Astiasarán, 2004; Ruiz-Capillas, Triki, Herrero, Rodríguez-Salas, & Jiménez-Colmenero, 2012). This has led to modifying traditional formulations to make them healthier. Numerous studies have reported new formulations for healthier reduced fat fermented sausages where partial fat replacement by lean meat is accompanied by the addition of other ingredients and the fatty acid profile is improved using vegetable and marine oils (individually or combined) as partial substitutes for animal fat (García, Dominguez, Galvez, Casas, & Selgas, 2002; Jiménez-Colmenero, 2007; Josquin, Linssen, & Houben, 2012; Mendoza, García, Casas, & Selgas, 2001; Muguerza et al., 2004; Muguerza, Gimeno, Ansorena, Bloukas, & Astiasaran, 2001; Pelser, Linssen, Legger, & Houben, 2007; Ruiz-Capillas et al., 2012; Valencia, Ansorena, & Astiasaran, 2006). As it is not easy to modify the lipid fraction in fermented sausage, these two strategies are not normally linked because of the difficulties involved in the manufacturing technology and the physicochemical characteristics of the products. For this reason, although these meat products contain high fat concentrations, relatively low amounts of non-meat fat are generally incorporated in comparison with other meat products (Jiménez-Colmenero, 2007).

Recently our group (Jiménez-Colmenero et al., in press) reported the nutritional, technological and sensorial viability of a dry fermented sausage reformulation process designed to produce better lipid compositions (reducing the fat content and improving the fatty acid profile) by replacing pork backfat by a healthier oil combination (olive, linseed and fish oils) stabilized in a konjac matrix. In the reformulated product ("chorizo") the total n-3 PUFA were around 1.12 g/100 g, of which approximately 0.95 g/100 g was alpha-linolenic acid (ALA) and 170 mg/100 g were long chain n-3 PUFA. This resulted in important fat and energy reductions (around 70% and 46% respectively), and is also linked to a very notable decrease in the n-6/n-3 ratio (11.25 versus 1.19), as compared with the normal fat product. In terms of energy contribution, the incorporation of the oil-in-konjac matrix in dry sausage reduces the

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proportion of calories from saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) and increases those provided by PUFA. These compositional modifications produced some changes in the sensorial appreciation (when compared to normal fat sausage), although they were still considered acceptable for the parameters related to appearance, flavour and juiciness (Jiménez-Colmenero et al., in press). Therefore the results for nutritional, technological and sensory properties showed that it is feasible to produce such low-fat n-3 PUFA dry sausage. However, other quality-related characteristics (including safety, shelf-life and physicochemical properties) need to be considered to obtain a clearer understanding of these products and a more accurate assessment of the suitability of this strategy for a healthier reformulation of dry sausages. Thus, the compositional changes during chilled storage affecting the product characteristics such as colour, texture, lipid oxidation, microbiological or biogenic amines formation must be evaluated.

The studies described in this paper were carried out to assess the influence of the reformulation process and chilled storage on the quality characteristics of dry sausages reformulated to produce better lipid composition (by reducing fat content and improving the fatty acid profile). The new products were produced by replacing the pork backfat with a healthier oil combination (olive, linseed and fish oils) stabilized in a konjac matrix. Weight loss, pH, water activity, colour, texture, lipid oxidation, microbiological changes and biogenic amines formation in low-fat, n-3 PUFA-enriched dry sausages were evaluated during the storage for 61 days at 2 °C. As far as the authors are aware, there are no references in the literature to similar research into reformulation strategies for new healthier-lipid dry sausages as reported in this paper.

2. Materials and methods

2.1. Materials, dry fermented sausages preparation and chilled storage

The ingredients and preparation procedures used for the manufacture of the konjac materials and dry "chorizo" sausage were reported by Jiménez-Colmenero et al. (in press). Briefly, fresh post-rigour pork meat (22.4, 5.0, 72.8% of protein, fat and moisture contents, respectively) and pork backfat (7.0, 88.5 and 3.2% of protein, fat, and moisture contents, respectively) were obtained from a local market. Konjac materials used as animal fat replacement were made with konjac flour from Trades S.A. (Barcelona, Spain), pre-gelled cornstarch (Amigel, Julio Criado, S.L. Madrid. Spain). i- carrageenan (Hispanagar S.A, Burgos, Spain) and $Ca(OH)_2$ (Panreac Química S.A., Barcelona, Spain). Olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain), linseed oil (Natursoy S.L., Alimentos Ecológicos, Castellterçol, Spain) and fish oil (Omevital 18/12 TG Gold, Cognis GMBH, Illertissen, Germany) were used to prepare the healthier oil combination which was prepared with 44.39% olive oil, 37.87% linseed oil and 17.74% fish oil and designed to produce a lipid material with a low proportion of SFA, high proportions of MUFA and PUFA (including long chain n-3 PUFA) and balanced n-6/n-3 PUFA and PUFA/SFA ratios (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010).

Other ingredients and additives used were sodium chloride (Panreac Química, S.A. Barcelona, Spain), sodium tripolyphosphate (STP) (Manuel Riesgo, S.A. Madrid, Spain), sodium nitrite (Fulka Chemie GMBH, Buchs, Germany), and two commercial prepared "choravi" (colour/flavour salts) and "curavi" (curing salts) from ANVISA (Arganda del Rey, Spain).

The three types of konjac materials were used in the dry fermented sausage formulation: one with no added oil (KG) and the other two incorporating different oil combination proportions (10% and 20%) (K10 and K20, respectively) into a konjac matrix (oil-in-konjac matrix). The four different dry fermented sausages were formulated using a similar amount of lean meat and the fat reduction level, were those described by Jiménez-Colmenero et al. (in press) (Table 1): a control sample, prepared with normal fat content (NF); a low fat sample (LFKG) produced by replacing 75% of added pork backfat by the same proportion of KG; and finally two more low fat samples (LFK10 and LFK20) formulated by substituting 90% and 100% of pork backfat by the same proportion of K10 and K20, respectively. The sausages were manufactured as described Jiménez-Colmenero et al. (in press). Briefly, first, the meat, backfat and konjac materials were minced at 15 mm (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy). The backfat and/or konjac materials (depending on the formulation) were thoroughly mixed manually with the meat and then minced again at 15 mm. This batter was homogenized for 60 s (MAINCA, Granollers, Barcelona, Spain), then half of the additives (choravi, curavi and NaCl), were added and the mixture homogenized again for 60 s. The remaining additives were then added and the mixture homogenized for 120 s more. The prepared sausage mixture was manually stuffed using a stuffer (MAINCA, Granollers, Barcelona, Spain) into 4 cm diameter collagen casing (Fibran S. A. Sant Joan de les Abadesses, Gerona, Spain). The sausages were hand-linked to standard sizes (22–23 cm), which were placed in a ripening cabinet (BINDER model KBF 240 Tuttlingen, Germany) programmed to operate under the following conditions: 48 h at 23 °C, 90% relative humidity (RH), then at 13 °C, 70–80% RH. The end of the ripening process was defined when weight loss (in relation to the initial weight) of the sausages reached 37–42%. The dry fermented sausages were packed in plastic bags (BB3050 Cryovac® Spain) (under aerobic conditions) and stored under refrigeration (2 ± 2 °C) for two months. Samples from each formulation were taken regularly for analysis to monitor how chilled storage affected the quality characteristics of these reformulated products.

2.2. Weight losses, pH and water activity

Weight losses of products during storage were evaluated as % of initial sample weight. Three sausages for each formulation were used for this determination.

The pH was determined (in sextuplicate) on a Radiometer model PHM 93 pH-meter (Meterlab, Copenhagen, Denmark) at room temperature on homogenates of meat products in water in a ratio 1:10 (w/v).

Water activity (a_w) was measured (in quadruplicate) in a Lab-Master- a_w instrument (model 1119977, Novasina AG, Lachen SZ, Switzerland) at 25 °C after removing the casing.

Table 1
Formulation (%) of dry fermented sausages.[a]

Samples	Meat	Backfat	Fat replacer		
			Konjac gel	Oil-in-konjac matrix	
				K10	K20
NF	73.9	18.5	–	–	–
LFKG	73.9	4.62	13.8	–	–
LFK10	73.9	2.20	–	16.3	–
LFK20	73.9	–	–	–	18.5

[a] Samples: NF, dry fermented normal fat sausage formulated with all pork fat; LFKG, dry fermented low-fat sausage reformulated replacing pork backfat by konjac gel; LFK10 y LFK20, dry fermented low-fat sausage reformulated replacing pork backfat by an oil-in-konjac matrix (K10 and K20) containing 10 and 20% of healthy oil combination (44.39% olive oil, 37.87% linseed oil and 17.74% fish oil), respectively. The following were also added to all samples: 5.5% of "choravi", 0.27% of "curavi" and 1.85% sodium chloride.

2.3. Texture profile analysis (TPA)

TPA, as described by Bourne (1978), was carried out using a TA.XT2i Stable Micro Systems Texture Analyser (Stable Microsystems Ltd., Surrey, England). Ten cores (diam = 20 mm, height = 20 mm) per sample were axially compressed to 50% of their original height. A 30 kg load cell was used at a crosshead speed of 1 mm/s. Attributes were calculated as follows: hardness (Hd) = peak force (*N*) required for first compression; cohesiveness (Ch) = ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp) = distance (mm) the sample recovers after the first compression; chewiness (Cw) = Hd × Ch × Sp (*N* × mm). Measurements were performed at 22 °C.

2.4. Lipid oxidation

Oxidative stability was evaluated by changes in thiobarbituric acid-reactive substances (TBARS). The procedure for measurement of TBARS was described by Delgado-Pando, Cofrades, Ruiz-Capillas, and Jiménez-Colmenero (2010). Briefly, the procedure was as follows: 5 g of each sample was homogenized in 35 ml of 7.5% trichloroacetic acid for 30 s at high speed in an Ultraturrax blender (Ika-Werke, GmbH & Co, Staufen, Germany). The blender sample was centrifuged (3000× *g*, 2 min) (Sorvall RTB6000B, DuPont, USA) and 5 ml of the supernatant was mixed with 5 ml of 20 mM thiobarbituric acid; finally the solution was mixed and kept in the dark for 20 h at 22 ± 1.5 °C. The pink colour formed was determined spectrophotometrically (Lambda 15UV/VIS spectrophotometer, Perkin–Elmer, USA) at 532 nm. A calibration curve was plotted with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO, USA) to obtain the malonaldehyde (MDA) concentration. The results were expressed as mg malonaldehyde/kg of sample. TBARS determinations were performed three times.

2.5. Microbiological analysis

Microbiological analysis of sausages was carried out by duplicated as follows: 10 g of each sample were taken and placed in a sterile plastic bag with 90 ml of peptone water (0.1%) with 0.85% NaCl. After 2 min in a stomacher blender (Stomacher Colworth 400, Seward, UK), appropriate decimal dilutions were pour-plated (1 mL) on the following media: Plate Count Agar (PCA) (Merck, Germany) for the total viable count (TVC) (30 °C for 72 h); De Man, Rogosa, Sharp Agar (MRS) (Merck, Germany) for lactic acid bacteria (30 °C for 3–5 days); and Violet Red Bile Glucose Agar (VRBG) (Merck, Germany) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of colony-forming units per gram (Log cfu/g).

2.6. Biogenic amines (BA) determination

Tyramine, phenylethylamine, histamine, putrescine, cadaverine, agmatine, triptamine, spermidine and spermine were determined in an extract prepared by blending 15 g of each sample (from at least two sausages) with 30 mL of 7.5% trichloroacetic acid in an omnimixer (Omni International, Waterbury.ct.USA) (20,000 rpm, 3 min) and centrifuged at 5000× *g* for 15 min at 4 °C in a desktop centrifuge (Sorvall RTB6000B, DuPont, USA). The supernatants were filtered through a Whatman n° 1 filter and passed back through a 0.22 μm Nylon filter (Millipore, Ireland). This filtrate was injected into an HPLC model 1022 with a Pickering PCX 3100 post-column system (Pickering Laboratories, Mountain View, Ca, USA) following the methodology described by Triki, Jiménez-Colmenero, Herrero, and Ruiz-Capillas (2012). The results are the mean of at least 3 determinations.

2.7. Statistical analysis

Data were analysed using SPSS Statistics 17.0 (SPSS Inc, Chicago, USA) for one-way and two-way ANOVA. Least squares differences were used for comparison of mean values among treatments and Tukey's HSD test to identify significant differences ($P < 0.05$) between formulations and storage times. The experiment was replicated twice.

3. Results and discussion

Lipid modification of meat products by replacing animal fat with other lipid sources has proved to be a good strategy to improve nutritional quality and to achieve the desired biochemical effects without the ingestion of supplements or changes in dietary habits. As one of the commonest foods in our diet, meats are an especially suitable vehicle for adding healthier lipids (Jiménez-Colmenero, 2007). Because of its technological and health properties, the use of konjac material as a fat analogue has been demonstrated to open up interesting possibilities for improving fat content in dry fermented sausages (Ruiz-Capillas et al., 2012). These possibilities include a new proposal to incorporate healthy oils into a konjac matrix as a strategy for improving the fat content of meat products, with additional health benefits linked to the presence of dietary fibre. In this context, a previous paper by Jiménez-Colmenero et al. (in press) reported the proximate composition, fatty acid profile and sensory analyses of dry fermented sausage designed and reformulated to produce better lipid compositions (reducing fat content and improving the fatty acid profile) by replacing pork backfat by a healthier oil combination (olive, linseed and fish oils) stabilized in a konjac matrix. These reformulated products presented an important fat and energy value reduction accompanied by n-3 PUFA enrichment with an associated very marked decrease in the n-6/n-3 ratio. The reformulation process also produced a decrease ($P < 0.05$) in all sensorial parameters, compared with the control sample, although in all the sausages, appearance, flavour and juiciness scored over the mean value.

The following sections deal with different aspects of the physicochemical properties, safety and shelf-life characteristics essential to obtain a clearer understanding of these new products.

3.1. Weight loss, pH and water activity

During the dry fermented sausage storage only minor weight losses were observed (lower than 1.5%), which were not affected ($P > 0.05$) by product formulation. Such low levels of weight loss can be attributed to the product packaging which limited the desiccation process during the storage. The pH values of the dry sausage were not affected ($P > 0.05$) by the sausage formulation and storage period; for this reason only the pH mean values were reported (NF: 5.00 ± 0.08; LFKG: 4.82 ± 0.05; LFK10: 4.89 ± 0.06; LFK20: 4.99 ± 0.07).

The initial water activity (a_w) values of the dry fermented sausages varied from 0.896, 0.872, 0.884 for LFKG, LFK10 and LFK20 samples, respectively, to the lowest ($P < 0.05$) value (0.856) for the control sausage (NF). These a_w values are within the normal range described for this fermented product (Herrero et al., 2007). No significant differences were observed during the storage.

3.2. Texture profile analysis

Composition changes may affect product characteristics such as texture during chilled storage, especially in products with a long

shelf life such as dry sausages. TPA parameters were affected ($P < 0.05$) by formulation and storage time, with interaction ($P < 0.05$) between the two factors (Table 2). Initially reducing fat by partial replacement of animal fat with konjac gel (NF versus LFKG) decreased ($P < 0.05$) hardness and increased ($P < 0.05$) cohesiveness, with no effect ($P > 0.05$) on springiness and chewiness. Reducing fat content by replacing pork backfat by konjac gel (Table 1) resulted on a product with similar protein content, but higher moisture proportion (Jiménez-Colmenero et al., in press); the higher moisture/protein ratio in low-fat sample (LFKG) helped to explain the softer structures in product matrix as compared with NF sample. In contrast to these findings, the formation of harder structures has been reported as fat content decreases in dry fermented sausages (Liaros, Katsanidis, & Bloukas, 2009; Muguerza, Fista, Ansorena, Astiasarán, & Bloukas, 2002; Muguerza et al., 2004). Ruiz-Capillas et al. (2012) also reported that as fat content decreased (and konjac gel levels increased) hardness and chewiness increased, while cohesiveness is reduced. This apparent discrepancy may be attributed to the variations in moisture/protein ratio of the products as the result of the different criteria used to establish the completion of the ripening process in both experiments (Jiménez-Colmenero et al., in press). Irrespective of the reformulation process, all low fat samples had similar initial TPA parameters, so that no effects of incorporating oils stabilized in a konjac matrix were observed. Josquin et al. (2012) reported a variation in firmness of Dutch-style sausages as a result of the incorporation of fish lipid (pure, pre-emulsified or encapsulated).

During chilled storage an increase ($P < 0.05$) in hardness occurred, and at the end of the test period this parameter showed increases of around 50–60% in the case of NF, LFK10 and LFK20 samples and even higher in the LFKG sample (Table 2). Cohesiveness and springiness (except for LFK20) decreased ($P < 0.05$) over the storage period. Significant changes in springiness only occurred when the animal fat was replaced with konjac gel (LFKG sample), with an increase ($P < 0.05$) on day 14 of storage and with no later modifications ($P > 0.05$) (Table 2). Since low levels of weight loss over storage were observed, the changes in TPA parameters were not related to quantitative modifications in product composition. Rubio et al. (2007) reported that hardness, springiness, cohesiveness and chewiness of dry fermented sausage increased over the whole storage period (150 days).

3.3. Lipid oxidation

One of the potential problems derived from healthier fat meat product formulations is an acceleration of the oxidative process with implications for quality and health. TBARS values were affected ($P < 0.05$) by formulation and storage, with interaction ($P < 0.05$) between both factors (Table 3). Initially low levels of lipid oxidation were observed, although fat reduction through the partial replacement of animal fat with konjac gel (NF versus LFKG) presented the lowest ($P < 0.05$) TBARS values, while the incorporation of oil-in-konjac led to an increase ($P < 0.05$) in TBARS values. Independently of the formulation, initial oxidation levels were similar to those reported by other authors in this type of product (Bloukas, Paneras, & Fournitzis, 1997; García-Iníguez de Ciriano et al., 2010). Lipid oxidation increased throughout the storage period in all samples; in NF and LFKG a small but significant change occurred in TBARS values, but the oxidative process was significantly greater ($P < 0.05$) in dry fermented sausage containing oil-in-konjac (LFK10 and LFK20), with TBARS levels four times higher at the end of the storage period (Table 3). This behaviour can be attributed to the greater susceptibility to lipid oxidation of unsaturated (particularly polyunsaturated) fatty acids, present in high quantities when animal fat was partially replaced by oil-in-konjac matrix (Jiménez-Colmenero et al., in press). The oxidation level found in this study at the end of storage time for all-pork fat samples (NF and LFKG) (TBARS values around 0.50 mg/kg) can be considered low for this kind of product (Bloukas et al., 1997), while the rate observed in the samples containing oil-in-konjac matrix was similar to that in Greek fermented sausage formulated with olive oil (Bloukas et al., 1997; Muguerza et al., 2002).

Lipid oxidation in healthier fat meat product formulations varies according to the nature of the product, the type, amount and means of addition of non-meat fats, and the antioxidative system used to control rancidity development (Jiménez-Colmenero, 2007). No oxidation problems have been detected in partial substitution of pork backfat by olive oil (Bloukas et al., 1997; Muguerza et al., 2001), linseed oil (Ansorena & Astiasarán, 2004; Valencia et al., 2006), soy oil (Muguerza et al., 2002) or oil from microalgae (Valencia, Ansorena, & Astiasaran, 2007) in fermented meat products. Replacing beef fat with olive oil has been reported to favour lipid oxidation in traditional Turkish dry fermented sausage

Table 2
TPA parameters of dry fermented sausage during chilled storage.[a]

Parameters	Samples	Days of storage			
		0	14	33	61
Hardness (N)	NF	39.31 ± 3.35a2	53.32 ± 5.87b2	52.73 ± 4.91b3	58.39 ± 4.54b3
	LFKG	30.22 ± 5.00a1	47.82 ± 4.33b2	51.13 ± 4.79b23	64.03 ± 5.06c3
	LFK10	31.81 ± 3.50a1	33.65 ± 5.41b1	39.31 ± 3.27c1	49.09 ± 3.43d2
	KLF20	27.47 ± 3.02a1	36.97 ± 3.72b1	35.19 ± 4.40b1	45.77 ± 3.30c2
Cohesiveness (dimensionless)	NF	0.53 ± 0.06b1	0.46 ± 0.04a1	0.46 ± 0.04a1	0.45 ± 0.02a1
	LFKG	0.59 ± 0.03c2	0.54 ± 0.02b2	0.52 ± 0.02b2	0.46 ± 0.08a1
	LFK10	0.56 ± 0.01b12	0.58 ± 0.02b2	0.55 ± 0.02b2	0.48 ± 0.03a1
	KLF20	0.59 ± 0.02b2	0.56 ± 0.03b2	0.47 ± 0.03a1	0.57 ± 0.04b2
Springiness (mm)	NF	0.63 ± 0.02b1	0.53 ± 0.04a1	0.51 ± 0.03a1	0.54 ± 0.04a1
	LFKG	0.68 ± 0.06b12	0.59 ± 0.03a2	0.59 ± 0.06a12	0.60 ± 0.03a2
	LFK10	0.67 ± 0.04b12	0.64 ± 0.03ab2	0.63 ± 0.04ab2	0.59 ± 0.05a2
	KLF20	0.70 ± 0.03b2	0.61 ± 0.06a2	0.67 ± 0.02b3	0.58 ± 0.04a1
Chewiness (Nxmm)	NF	13.26 ± 2.23a1	13.17 ± 1.95a12	14.55 ± 1.41a12	13.20 ± 0.83a1
	LFKG	12.05 ± 1.87a1	15.32 ± 2.08b2	16.80 ± 2.02b2	17.63 ± 2.37b2
	LFK10	11.84 ± 1.33a1	12.40 ± 2.08a1	13.66 ± 1.30a1	13.98 ± 1.33a1
	KLF20	11.30 ± 1.72a1	12.46 ± 1.59a1	12.31 ± 1.12a1	13.27 ± 1.47a1

Means ± standard deviation. Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).

[a] Samples: NF, dry fermented normal fat sausage formulated with all pork fat; LFKG, dry fermented low-fat sausage reformulated replacing pork backfat by konjac gel; LFK10 y LFK20, dry fermented low-fat sausage reformulated replacing pork backfat by an oil-in-konjac matrix containing 10 and 20% of healthy oil combination (44.39% olive oil, 37.87% linseed oil and 17.74% fish oil), respectively.

Table 3
Thiobarbituric acid-reactive substances (TBARS, mg MDA/kg) of dry fermented sausages during chilled storage.[a]

Samples	Days of storage			
	0	14	33	61
NF	0.33 ± 0.12a2	0.44 ± 0.01b12	0.45 ± 0.01b1	0.54 ± 0.01c2
LFKG	0.23 ± 0.02a1	0.37 ± 0.02b1	0.46 ± 0.03c1	0.45 ± 0.02c1
LFK10	0.44 ± 0.02a3	0.50 ± 0.01a2	0.98 ± 0.01b2	1.98 ± 0.03c3
LFK20	0.54 ± 0.02a4	0.60 ± 0.02a3	1.09 ± 0.03b3	2.25 ± 0.02b4

Means ± standard deviation. Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).

[a] Samples: NF, dry fermented normal fat sausage formulated with all pork fat; LFKG, dry fermented low-fat sausage reformulated replacing pork backfat by konjac gel; LFK10 y LFK20, dry fermented low-fat sausage reformulated replacing pork backfat by an oil-in-konjac matrix containing 10 and 20% of healthy oil combination (44.39% olive oil, 37.87% linseed oil and 17.74% fish oil), respectively.

(Kayaardi & Gök, 2003). Oxidation has been reported in dry fermented sausage containing fish oil extract during curing (Muguerza et al., 2004), but Valencia et al. (2006) found no signs of oxidation in a product enriched with n-3 PUFAs from fish oil in the presence of antioxidants (BHA + BHT). Pelser et al. (2007) have reported that the addition of canola oil and encapsulated flaxseed oil in Dutch-style fermented sausages did not reduce shelf life in terms of lipid oxidation, but that the addition of flaxseed oil and encapsulated fish oil increased lipid oxidation during storage. In Dutch-style fermented sausages which were manufactured with 15% and 30% of pork back-fat substitution with the use of pure or commercial encapsulated fish oil, the addition of pure fish oil resulted in more advanced lipid oxidation; whereas adding commercial encapsulated oil resulted in less advanced lipid oxidation, compared to the control products (Josquin et al., 2012).

3.4. Microbiological analysis

The microbiological aspects of meat products must be considered in relation to food quality and safety. The products present high initial microbial counts (>8 Log cfu/kg) of total viable counts and lactic acid bacteria (Table 4), which are maintained during chilled storage without any clear appreciable relationship with product formulation. These levels are considered characteristic of this type of fermented products (Bover-Cid et al., 2009; González-Fernández, Santos, Jaime, & Rovira, 2003; Latorre-Moratalla et al., 2008; Maijala, Eerola, Lievonen, Hill, & Hirvi, 1995; Ruiz-Capillas et al., 2012). In the case of enterobacteria, the initial levels were below 4 Log cfu/g, with higher ($P < 0.05$) enterobacterial counts observed in LFK10 and LFK20 sausages, which contain oil in the reformulation. In contrast, LFKG and control samples presented a similar level ($P > 0.05$) of enterobacteria. After only 14 days of storage, the LFK10 sample maintained high enterobacterial levels. Other authors, however, have not observed microorganism level variations in products formulated with different fat content, even using oil-in-water emulsion as animal fat replacement (Koutsopoulos, Koutsimanis, & Bloukas, 2008; Liaros et al., 2009; Ruiz-Capillas et al., 2012). However, in cooked products such as frankfurter-type sausage, the use of oil-in-water emulsion (stabilized with sodium caseinate and soy protein isolate) to improve fat content has been observed to favour the microorganism counts, although the effect depends more on the type of oil-in-water emulsion than on fat levels (Delgado-Pando et al., 2011).

3.5. Biogenic amines

Biogenic amines (BA) are biologically active low-molecular-weight basic nitrogenous compounds, which could represent a potential public health and quality food concern. Their formation can be conditioned by changes in product composition. Many of the factors promoting or inhibiting biogenic amines formation (raw material characteristics, substrate source, reaction of the medium, microbiological aspects) can be considerably altered by meat product reformulation (Ruiz-Capillas & Jiménez-Colmenero, 2004). Although BA formation is a very important aspect in fermented meat products, improved fat content reformulation processes are not generally accompanied by studies of how this influences BA content, and very little related information is available (Ruiz-Capillas et al., 2012). For quality and safety reasons, the BA formation in new products must be considered.

Biogenic amines contents ($P < 0.05$) were affected by formulation and storage, with interaction ($P < 0.05$) between both factors (Table 5). Initially, the biogenic amines which presented the highest concentrations were tyramine, cadaverine, putrescine and the physiological amine, spermine. These levels are related to the dry sausage ripening process, which favours the formation of these compounds and to a lesser extent the physiological amines (Maijala et al., 1995; Ruiz-Capillas & Jiménez-Colmenero, 2004; Ruiz-Capillas et al., 2012). Reformulation affected the presence of some biogenic amines, such as tyramine and cadaverine, typical amines in fermented products (along with putrescine), with the highest levels ($P < 0.05$) observed in the LFKG and LFK10 samples, in the case of cadaverine, and in LFKG and LFK20 in the case of tyramine (Table 5). Similarly, the highest histamine values ($P < 0.05$) were

Table 4
Microbiological counts (Log cfu/g) of dry fermented sausages during chilled storage.[a]

Microorganisms	Samples	Days of storage			
		0	14	33	61
Total viable count	NF	8.54 ± 0.09a1	8.45 ± 0.12a1	8.53 ± 0.05a1	8.94 ± 0.20b2
	LFKG	8.79 ± 0.22b12	8.74 ± 0.03b2	8.76 ± 0.00b1	8.42 ± 0.08a1
	LFK10	8.59 ± 0.05a12	8.67 ± 0.01a12	8.70 ± 0.10a1	8.75 ± 0.04a2
	LFK20	8.86 ± 0.08a2	8.88 ± 0.06a2	8.78 ± 0.01a1	8.71 ± 0.08a12
Lactic acid bacteria	NF	8.48 ± 0.00a1	8.52 ± 0.06a1	8.63 ± 0.09a1	9.02 ± 0.08b2
	LFKG	8.92 ± 0.10ab2	8.80 ± 0.01ab2	8.72 ± 0.01a1	8.97 ± 0.10b12
	LFK10	8.63 ± 0.05a1	8.78 ± 0.08a2	8.64 ± 0.01a1	8.78 ± 0.11a1
	LFK20	9.11 ± 0.10b2	8.87 ± 0.03a2	8.77 ± 0.02a1	8.81 ± 0.03a1
Enterobacteriaceae	NF	2.65 ± 0.07b1	2.27 ± 0.05b2	1.24 ± 0.34a1	1.89 ± 0.41ab1
	LFKG	2.30 ± 0.00b1	1.00 ± 0.00a1	1.00 ± 0.00a1	1.52 ± 0.74ab1
	LFK10	3.46 ± 0.15b2	3.20 ± 0.12b3	3.46 ± 0.23b2	2.45 ± 0.52a1
	LFK20	3.85 ± 0.42b2	3.31 ± 0.11b3	1.35 ± 0.49a1	1.72 ± 0.17a1

Means ± standard deviation. Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).

[a] Samples: NF, dry fermented normal fat sausage formulated with all pork fat; LFKG, dry fermented low-fat sausage reformulated replacing pork backfat by konjac gel; LFK10 y LFK20, dry fermented low-fat sausage reformulated replacing pork backfat by an oil-in-konjac matrix containing 10 and 20% of healthy oil combination (44.39% olive oil, 37.87% linseed oil and 17.74% fish oil), respectively.

Table 5
Biogenic amines (mg/kg) of dry fermented sausages during chilled storage.[a]

Biogenic amines	Samples	Days of storage			
		0	14	33	61
Tyramine	NF	71.98 ± 1.06a1	86.87 ± 1.67b1	91.12 ± 6.72c1	107.53 ± 1.79d1
	LFKG	94.84 ± 1.16a2	108.29 ± 2.21b2	122.43 ± 0.57c3	129.04 ± 1.84d2
	LFK10	68.63 ± 3.56a1	190.25 ± 4.40b3	198.20 ± 0.67c4	225.89 ± 0.76d3
	LFK20	90.92 ± 0.76a2	106.10 ± 1.59b2	112.14 ± 0.59c2	108.14 ± 0.59bc1
Histamine	NF	1.22 ± 0.10a3	1.29 ± 0.19ab12	1.48 ± 0.01b3	2.09 ± 0.24c2
	LFKG	0.17 ± 0.01a1	1.07 ± 0.04c1	0.77 ± 0.02b1	2.91 ± 0.21d4
	LFK10	0.59 ± 0.15a2	2.11 ± 0.26c3	1.09 ± 0.07b2	2.54 ± 0.03d3
	LFK20	0.38 ± 0.02a12	1.42 ± 0.21c2	0.79 ± 0.01b1	1.76 ± 0.02d1
Putrescine	NF	11.24 ± 0.68a12	14.86 ± 1.08b1	15.97 ± 0.09b1	26.29 ± 1.55c1
	LFKG	11.23 ± 1.53a12	20.05 ± 0.05b2	23.66 ± 0.39c2	30.66 ± 1.52d2
	LFK10	10.81 ± 0.03a1	26.50 ± 0.14b3	29.19 ± 0.58c3	30.52 ± 1.22c2
	LFK20	12.59 ± 0.11a2	30.48 ± 1.91b4	37.12 ± 0.25c4	38.35 ± 0.98c3
Cadaverine	NF	25.15 ± 1.25a1	25.97 ± 2.02a1	36.88 ± 0.07b2	47.36 ± 1.56c2
	LFKG	135.90 ± 0.50a3	157.22 ± 0.82b3	195.80 ± 2.20c4	210.30 ± 0.70d4
	LFK10	108.42 ± 2.88a2	125.00 ± 5.66b2	161.20 ± 4.41c3	192.36 ± 1.49d3
	LFK20	24.82 ± 2.30a1	27.24 ± 0.20ab1	30.26 ± 0.04bc1	33.07 ± 1.22c1
Tryptamine	NF	ND	0.16 ± 0.01a1	0.23 ± 0.01a1	0.42 ± 0.02a1
	LFKG	1.37 ± 0.11a1	12.11 ± 0.47b4	12.77 ± 0.26c4	13.98 ± 0.02d4
	LFK10	ND	0.27 ± 0.02a2	0.45 ± 0.05b2	0.72 ± 0.02c2
	LFK20	ND	0.60 ± 0.16a3	0.80 ± 0.00ab3	1.16 ± 0.15b3
Agmatine	NF	0.03 ± 0.01a1	0.56 ± 0.01b2	0.70 ± 0.10c1	0.78 ± 0.02c1
	LFKG	ND	0.73 ± 0.00a4	1.75 ± 0.02b4	2.33 ± 0.07c3
	LFK10	ND	0.50 ± 0.00a1	1.43 ± 0.05b3	1.79 ± 0.13c2
	LFK20	ND	0.62 ± 0.01a3	0.90 ± 0.03b2	2.35 ± 0.06c3
Spermidine	NF	2.43 ± 0.39b1	1.85 ± 0.15a1	3.12 ± 0.28c3	1.70 ± 0.15a1
	LFKG	2.70 ± 0.32b1	2.15 ± 0.02a2	2.89 ± 0.07b3	2.22 ± 0.06a2
	LFK10	2.44 ± 0.02b1	1.85 ± 0.05a1	1.59 ± 0.08a1	1.70 ± 0.03a1
	LFK20	2.43 ± 0.05b1	1.96 ± 0.07a1	2.09 ± 0.22a2	2.20 ± 0.05ab2
Spermine	NF	31.45 ± 3.69a1	33.66 ± 0.99ab1	35.67 ± 1.36bc1	38.02 ± 1.29c1
	LFKG	37.98 ± 3.76ab23	38.08 ± 1.63ab2	36.66 ± 0.40a12	40.11 ± 1.23b12
	LFK10	39.08 ± 0.88a3	41.20 ± 0.96ab3	43.00 ± 0.78b3	43.46 ± 1.27b2
	LFK20	37.16 ± 0.84a2	38.57 ± 0.56a2	39.80 ± 0.71a23	39.91 ± 0.12a1

Means ± standard deviation. Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).
ND: Not detected.
[a] Samples: NF, dry fermented normal fat sausage formulated with all pork fat; LFKG, dry fermented low-fat sausage reformulated replacing pork backfat by konjac gel; LFK10 y LFK20, dry fermented low-fat sausage reformulated replacing pork backfat by an oil-in-konjac matrix containing 10 and 20% of healthy oil combination (44.39% olive oil, 37.87% linseed oil and 17.74% fish oil), respectively.

observed in the normal fat sample (NF) as compared with reformulated (low-fat) dry sausages. The opposite effect was observed for spermine, with NF presenting lower levels than the reformulated samples without affecting spermidine contents. Minor differences were observed in putrescine, tryptamine and agmatine, although these levels cannot be considered relevant in quantitative terms.

The biogenic amines concentrations generally increased ($P < 0.05$) with storage time (mainly in tyramine, histamine, putrescine and cadaverine), although the quantity varied with formulation and storage period (Table 5). No significant or quantitatively relevant changes ($P > 0.05$) were observed for histamine, tryptamine (except for LFKG), agmatine, spermidine and spermine (Table 5). At the end of storage, the amines which presented the highest concentrations were tyramine and cadaverine, followed by spermine and putrescine. LFKG and LFK10 samples presented the highest levels of tyramine and cadaverine, mainly of tyramine (226 mg/kg) in LFK10 dry sausage, and of cadaverine (210 mg/kg) in LFKG sausage. In contrast to these variations, both the low fat sample with the highest level of oil in the formulation (LFK20) and normal fat dry sausage (NF) presented similar concentrations ($P > 0.05$) of these amines.

The results obtained do not show a clear relationship between biogenic amines formation and fat improvement strategy, both when replacing pork backfat by konjac gel (reducing fat content), or incorporating healthier oil stabilized in a konjac matrix (fat reduction with added healthier oil combination). The microbiological results (Table 5) showed that biogenic amines formation may be related to the microbial population, but at a qualitative rather than quantitative level. Various authors (Bover-Cid et al., 2009; Maijala et al., 1995; de las Rivas et al., 2008; Roig-Sagués & Eerola, 1997) mentioned the existence of strain specificity, so that in a species at the same microorganism level there may be selective growth of strains with different biogenic amines production capacities. In the case of tyramine, where levels are related to lactic acid bacteria growth (de las Rivas et al., 2008), some authors have indicated that for example the growth of *Lactobacillus sakei* does not produce biogenic amines (Bover-Cid, Izquierdo-Pulido, & Vidal Carou, 2001; Roig-Sagués & Eerola, 1997). Similarly, for cadaverine and putrescine formation, which are related to enterobacterial growth (Bover-Cid et al., 2009), a similar phenomenon to that described above may have occurred.

Tyramine has been described as the predominant amine, followed by the diamines (putrescine and cadaverine) in dry "chorizo" sausage with a wide variability observed of these diamines (Latorre-Moratalla et al., 2008). Depending on the starters used in the formulation, some studies have reported that putrescine levels were higher than cadaverine's (González-Fernández et al., 2003; Ruiz-Capillas, Jiménez-Colmenero, Carrascosa, & Muñoz, 2007). In general terms, the biogenic amines levels observed in this study were similar to those observed by other authors in this type of products (Bover-Cid et al., 2001; Miguélez-Arrizado, Bover-Cid, Latorre-Moratalla, & Vidal-Carou, 2006; Ruiz-Capillas & Jiménez-Colmenero, 2004; Ruiz-Capillas, Triki, Herrero, & Jiménez-Colmenero, 2011) and they are not considered to cause a risk for the consumer. Although some authors have reported that toxic

tyramine amount for a normal person is considered between 700 and 800 mg/kg (Ruiz-Capillas & Jiménez-Colmenero, 2004), others mentioned 125 mg/kg of tyramine as a toxic level (Vidal-Carou, Izquierdo, Matín-Morro, & Marine-Font, 1990). However, in normal circumstances, by detoxication mechanisms, the human body is able to quickly detoxify histamine and tyramine absorbed from foods with the enzymes monoamine oxidase (MAO; EC 1.4.3.4), diamine oxidase (DAO; EC 1.4.3.6), and polyamine oxidase (PAO; EC 1.5.3.11) (Bardócz, 1995). Nevertheless, the toxicity of these amines in the body depends on the efficiency of the body's own detoxification system (Bardócz, 1995).

4. Conclusion

The new reformulation procedure applied to improve the fat content of dry fermented sausages, both by reducing the fat proportion and improving the fatty acid profile, showed that the increase in unsaturated lipid levels increased lipid oxidation susceptibility during dry fermented sausage chilling storage. However, no limitations related to microbiological aspects and biogenic amines formation were found as an effect of the formulation strategy and chilled storage. The microbiological and biogenic amines studies did not show any clear relationship between these parameters and fat improvement strategy, both when replacing pork backfat by konjac gel (reducing fat content), or incorporating healthier oil stabilized in a konjac matrix (fat reduction with addition of a healthier oils combination).

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IV.3. INFLUENCIA DEL CAMBIO DE COMPOSICIÓN DEL MERGUEZ EN LA FORMACIÓN DE AMINAS BIÓGENAS.

VI.3.1. Effect of preformed konjac gels, with and without olive oil, on the technological attributes and storage stability of merguez sausage

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Effect of preformed konjac gels, with and without olive oil, on the technological attributes and storage stability of merguez sausage

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ABSTRACT

In order to improve the fat content of fresh sausages (merguez), the effects of both reducing beef fat level (by konjac gel-KG) and incorporing olive oil (in a konjac matrix-OKM) on nutritional, quality characteristic and refrigerated storage stability were studied. Fat reductions in merguez sausages of between 53 and 76% were achieved when beef fat was replaced with KG; the proportion reached 34–49% using OKM as a beef fat replacer, where 23 to 36% of total fat in the merguez was from olive oil. The merguez contained substantial amounts of some minerals (Mg and Fe). Sensory analysis revealed no significant differences between the control and the reformulated products, which had relatively low levels of lipid oxidation. Shelf life and biogenic amines of merguez sausage were not affected by formulation during refrigerated storage. Therefore, the use of konjac materials as fat replacers could reduce total caloric energy by replacing/reducing beef fat and improving sausage formulation to achieve healthier merguez products.

1. Introduction

Merguez is a fresh sausage originating from North Africa that is widely consumed in different countries of the world including Europe and Asia. It is generally made of lean and fat beef (also with lamb or mutton) mixed with condiments stuffed into a lamb intestine. It is heavily spiced with chilli pepper or harissa, which gives it its characteristic hot and red color, as well as other spices such as fennel and mint. Merguez has a short shelf life even when stored at refrigerated temperature (Benkerroum, Daoudi, & Kamal, 2003). This raw strongly-spiced meat product is usually eaten grilled or with couscous; dried merguez is used to add flavor to typical Arabian and African dishes.

Fresh sausages of this kind usually present some negative health concerns related to their high fat (over 20%), energy (around 280–300 kcal/100 g) and salt (3.6%) contents, as well as the fatty acid profiles of the animal fat (ANSES, 2008). Like other meat products, merguez can be reformulated to achieve healthier lipid compositions. There is no doubt that dietary fat is needed as a metabolic energy source and a supplier of essential nutrients, but it must be consumed in moderation for reasons of human health. There is growing evidence associating dietary fat (quantity and type of fat) with chronic disorders such as ischaemic heart disease, some types of cancer, and obesity (WHO, 2003). Improving the fat content of foods has generally been seen as an important strategy to produce healthier products. This aspect is especially relevant to the processed meat industry because of the relatively high fat content in processed meats, including fresh and cooked sausages. Globally, meat accounts for about 8% of total energy availability, 18% of dietary protein, and 23% of dietary fat. Meat consumption is considerably higher in high-income countries—10% of total energy intake compared with 7% in low-income countries—(WCRF, 2007), although the amount of meat consumed in developing countries is increasing rapidly (Delgado, 2003). Therefore, healthier-lipid meat product formulations are important in all societies.

In order to improve fat content in meats, two different aspects must be considered: the reformulation of products containing less fat and a better fatty acid profile, by replacing the animal fat normally present with other fats more in line with health recommendations from plant or marine sources. Fat reduction in meat products is usually based on two main criteria: use of leaner meat raw materials and reduction of fat density (dilution) by adding water and other ingredients (Jiménez-Colmenero, 2007). These ingredients should assure a low calorie content and give the product the desired characteristics. One such ingredient is konjac (glucomannan)-based fat analogues, which open up interesting possibilities. Konjac is a neutral polysaccharide produced by *Amorphophallus konjac*, a plant native to East Asia, where it has been used since ancient times. The interest of this ingredient lies in its important technological properties (water retention capacity, gelling and thickening agent) and potential health implications (e.g. reducing cholesterol, insulin and glucose levels or its satiating and laxative effect) which offer great potential for application in food technology (Tye, 1991). Its use as a food additive is authorized in Europe (E-425), and it is classified as GRAS by the FDA. Konjac, added in different ways and concentrations, has been used to reduce fat in products such as frankfurters (Jiménez-Colmenero et al., 2010; Osburn & Keeton, 2004), bologna (Chin, Keeton, Miller, Longnecker, & Lamkey, 2000),

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fresh sausages (Osburn & Keeton, 1994), pork nuggets (Berry & Bigner, 1996) or pâtés (Delgado-Pando, Cofrades, Rodriguez-Salas, & Jiménez-Colmenero, 2011).

In order to improve fatty acid profiles, a variety of non-meat fats of plant and marine origin have been added to meat products as partial substitutes for meat fats, mainly pork or beef (Jiménez-Colmenero, 2007). Of vegetable oils, olive is the one that has received most attention, chiefly as a source of monounsaturated fatty acids (MUFAs). It has a high biological value attributed to a favorable mix of MUFAs and naturally-occurring antioxidants (vitamin E, vitamin K, carotenoids and various polyphenols: hydroxytyrosol, tyrosol, and oleuropein). Partial substitution of pork backfat by olive oil and high-oleic acid sunflower oil has been tried in meat products such as fresh, cooked and fermented sausages, beef patties, etc. (Jiménez-Colmenero, 2007; Koutsopoulos, Koutsimanis, & Bloukas, 2008; López-López, Cofrades, Yakan, Solas, & Jiménez-Colmenero, 2010). No studies have been conducted to combine 1) reduction of fat content and 2) alteration of fatty acid profile using konjac gels in fresh beef sausage (merguez).

One essential aspect of a strategy for achieving healthier lipid composition is the procedure used to incorporate the plant and marine oils in meat products. To that end, various technological options have been used, ranging from direct addition as liquid oils or as solids (including interesterified oils) to incorporation in encapsulated or pre-emulsified form or as part of plant ingredients (Jiménez-Colmenero, 2007). One such technological procedure that has yet to be explored is the incorporation of healthier oil into a konjac matrix and the use of this new ingredient as an animal fat replacer in meat products at the same time stabilizing the olive oil in the konjac gel matrix during formulation, processing and storage. Compared to other technological options, this approach to oil stabilization offers additional health benefits associated with the presence of konjac.

Therefore, the objective of this research was to evaluate the nutritional consequences, quality characteristics and storage stability (shelf life) of merguez (fresh beef sausage) produced by a reformulation process designed to improve fat content, by both reducing fat content (replacing the beef fat with konjac gel) and incorporating olive oil (replacing the beef fat with olive oil stabilized in a konjac matrix).

2. Materials and methods

2.1. Materials and konjac gel preparation

Fresh post-rigor beef (20.6%, 6.1%, 71.7% protein, fat and moisture contents respectively) and beef fat (10.0%, 46.1% and 37.3% protein, fat, and moisture contents respectively) were obtained from a local market, minced (15 mm diam. hole mincer plate) (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy), and frozen at −20 °C. Frozen storage did not exceed 14 days.

The following ingredients were used: konjac flour (glucomannan 83%, 120 mesh) from Trades S.A. (Barcelona, Spain), pre-gelled cornstarch (Amigel, Julio Criado, S.L. Madrid. Spain). i-carrageenan (Hispanagar S.A, Burgos, Spain) and Ca(OH)2 (Panreac Química S.A., Barcelona, Spain). Other ingredients and additives used were olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain), sodium chloride (Panreac Química, S.A. Barcelona, Spain) and various condiments and spices including coriander (Naturel, Conditionnement de produits agricoles, El Sahlin, Tunisia), fennel (Kamy S.A. (Nabeul) Tunisia), paprika and hot pepper (Jose Mª Fuster Hernandez S.A., Murcia, Spain), mint (DUCROS, Mac Cormick S.A., Spain) and a commercial preparation of Harissa (Ferrero, TUCAL S.A., Manouba, Tunisia). Harissa is one of the ingredients most commonly used in the Maghreb countries (especially Tunisia) to prepare certain foods, particularly meat products. Ingredients commonly include red hot pepper, garlic, coriander, caraway and salt.

Two types of konjac materials (fat analogues) were prepared: one a konjac gel (KG) and another with olive oil added to the konjac matrix (oil-in-konjac matrix/OKM). KG was prepared as described by Osburn and Keeton (2004) with modifications (Jiménez-Colmenero et al., 2010). Briefly, konjac flour (5.0%) was homogenized (Stephan Universal Machine UM5, Stephan Machinery GmbH and Co., Hameln, Germany) with 64.8% of the water for 3 min, left to rest for 5 min then homogenized for a further 3 min. The i-carrageenan (1.0%) was then added and the mixture homogenized again for 3 min. The pre-gelled cornstarch powder (3.0%) was dispersed in 16.2% of the water and homogenized with the mixture of konjac flour and i-carrageenan, left to rest for 5 min then homogenized for a further 3 min. The mixture was cooled to 10 °C, then 10% of a Ca(OH)2 solution (1%) was added with gentle stirring at room temperature.

Oil-in-konjac matrix (OKM) was prepared in the same way as KG, except that 20% w/w of olive oil was added just after addition of i-carrageenan and the mixture was homogenized for 3 min. In both types of konjac materials (KG, OKM) the formulation maintained the same proportions of the components used to prepare them with respect to the water base (hence not including the added oil). The preparation conditions and the technological viability of adding these proportions of oils to konjac materials (OKM) were established earlier. KG and OKM were placed in suitable containers, covered, manually overpressured to eliminate air and stored at 2 ± 2 °C until used (within 24 h of preparation). Both ingredients were prepared in duplicate.

2.2. Experimental design and merguez sausage manufacture

Merguez sausages were designed and formulated to improve fat content, using similar amounts of lean meat since fat was reduced by replacing the beef fat with the same proportion of two fat analogues (konjac materials). Five treatment formulations were evaluated (Table 1): a control formulation (C) prepared with 20% fat content, two formulations in which 75 and 100% of the beef fat was replaced by the same portion of KG (fat analogue). Thus, both reformulated products contained less fat than the control, but in these three formulations (C, 75/KG and 100/KG) all the fat was animal fat (from beef). Finally another two formulations (75/OKM and 100/OKM) were prepared in which 75% and 100% of the beef fat was replaced respectively by the same proportion of OKM (fat analogue); in this case the reformulated sausages contained higher fat levels than 75KG and 100KG due to the olive oil present in the OKM. This means that the animal fat was partially replaced by olive oil. According to the experimental design, the resulting products would have different fat levels.

The meat and fat were thawed before use (18 h at 2 ± 2 °C). The sausages were made as follows. Firstly meat, fat and konjac materials were minced together to a 15 mm particle size (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy) and placed in a mixer (MAINCA, Granollers, Barcelona, Spain). Half of the water and additives (Table 1) were added and sample mixed for 1 min. The other half of the additives

Table 1
Formulation (%) of merguez sausages.

	Beef meat	Beef fat	KG	OKM
C	55.00	29.00	–	–
75/KG	55.00	7.25	21.75	
75/OKM	55.00	7.25	–	21.75
100/KG	55.00	–	29.00	
100/OKM	55.00	–	–	29.00

Sample denomination: C—control sample (all beef fat) prepared with normal fat content; 75/KG and 100/KG—sausages prepared replacing 75% and 100% respectively of beef fat with the same proportion of KG (konjac gel); 75/OKM and 100/OKM—sausage prepared replacing 75% and 100% of beef fat respectively with the same proportion of OKM (oil-in-konjac matrix, as konjac material containing 20% of olive oil).
All samples also contain: 10.7% of water, 1.4% NaCl, 0.5 coriander, 0.8% fennel, 0.2% hot pepper, 0.2% paprika, 0.2% mint and 2.0% harissa.

was then added and the whole mixed again for 2 min. The final temperature of the meat batter was between 3 and 6 °C. The prepared sausage mixture was immediately stuffed, using a stuffer (MAINCA, Granollers, Barcelona, Spain), into 22 mm-diametre natural lamb casings (Type C-20/22 Julio Criado Gómez S.A. Spain). Sausages were handlinked to standard sizes of 10 ± 2 cm and the resulting strings of sausages were covered with plastic and placed in a room at 2 °C overnight for ingredient equilibrium (stabilization). After that, the sausages were placed on EPS trays (Type 89 white SPT—Linpac Packaging Pravia, S.A. N R.G.S. Spain), covered with oxygen-permeable cling film (LINPAC Plastics, Pontivy, France) in aerobic conditions and kept at 2 °C. Samples from each formulation were taken for analysis (days 0, 3, 5 and 7) to monitor the effect of storage on quality characteristics. Each formulation was prepared in duplicate.

2.3. Proximate analysis

Moisture and ash contents were determined (AOAC, 2005) in triplicate in all formulations. Protein content was measured in quadruplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St. Joseph, MI, USA). Fat content was evaluated in triplicate according to Bligh and Dyer (1959). Carbohydrates were estimated taking into account ingredient composition and formulation.

2.4. Mineral contents

Mineral content was determines as reported by Serrano et al. (2005). Briefly, formulations were ashed in triplicate in a furnace, with temperature gradients between 105 and 500 °C. The ash was dissolved in 2 ml concentrated nitric acid and diluted to 100 ml with Milli-Q water. The minerals were determined with an atomic absorption spectrophotometer (Perkin–Elmer, Model 5100, Norwalk, Connecticut. USA). A hollow cathode lamp was used to determine Ca, Mg, Fe, Na and K were analyzed by atomic emission (without a lamp). The analytical curve was determined for each element.

2.5. Sensory evaluation

Merguez sausages were assessed by a 20 member panel. Various prior training sessions on the product and terminology were conducted to familiarize the panelists with the merguez product. The training sessions were carried out with commercial merguez sausages bought in a Tunisian supermarket (Tunis City, Géant, S.A.) and various merguez sausages (similar to commercial merguez) manufactured in the laboratories. For sensory testing, merguez sausages were heated in porcelain dishes for 2 min (60 s on each side) then cut into small portions, placed on plates and served to the panellists. These were asked to evaluate the following parameters on a scale from 0 to 10: juiciness (0: not juicy, 10 very juicy), firmness (0 soft, 10 hard) and general acceptability (0 dislike, 10 like). The Sensory analysis was carried out three times for the different samples and at the start of storage.

2.6. Water and fat binding properties

The effects of reformulation on water and fat binding properties were evaluated in different conditions. Processing weight was estimated as weight loss occurring at 2 °C (overnight) prior to packaging and storage and expressed as % of initial sample weight. Three determinations for each sample were carried out.

For each storage time (except for day 0) the formulations were tempered for 20 min (at room temperature) and then removed from their packaging. The exudates were drained, the surface was gently dried with paper towels, and the sausages were weighed. Weight loss during storage (purge loss, PL) was evaluated in quadruplicate and expressed as a percentage of the initial weight.

To study water and fat binding properties associated with cooking, formulations (around 32 g, weighed exactly) were placed in containers (27 mm diameter) and heated (70 °C/30 min) in a water bath (GRANT, OLS 200, Grant instruments, Cambridge, Ltd., England). When heating was complete, the containers were opened and left to stand upside down (for 30 min) to release the exudate onto a plate that had been previously weighed. Cooking loss (CL) was expressed as % of initial sample weight. Water loss (WL) was determined as weight loss after heating, the total fluid released (on the plate used for cooking loss) for 16 h at 105 °C in a drying oven (Model IDL-AI-36, Labolan SL, Navarra, Spain) and was expressed as % of initial sample weight. Fat loss (FL) was calculated as the difference between CL and WL. Three determinations for each sample were carried out.

2.7. Lipid oxidation

Oxidative stability was evaluated from changes in thiobarbituric acid-reactive substances (TBARS). The procedure for measurement of TBARS was based on methods used by López-López et al. (2010). Briefly, the procedure was as follows: 5 g of each sample was homogenized in 35 ml of 7.5% trichloroacetic acid for 90 s at high speed in an Ultraturrax blender (Ika-Werke, GmbH & Co, Staufen, Germany). The blender sample was centrifuged (3000 *g*, 2 min, Solvall BA, RTB6000B, Dupont, USA) and 5 ml of the supernatant was mixed with 5 ml of 20 mM thiobarbituric acid; finally the solution was mixed and kept in the dark for 20 h at 20 ± 1.5 °C. Pink formation was measured spectrophotometrically (Lambda 15UV/VIS spectrophotometer, Perkin-Elmer, USA) at 532 nm. A calibration curve was plotted with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO, USA) to measure the malonaldehyde (MDA) concentration and results were expressed as mg malonaldehyde/kg of sample. TBARS determinations were performed three times.

2.8. Color measurement and pH

Color (CIE-LAB tristimulus values, lightness, L*; redness, a* and yellowness, b*) was evaluated on a Chroma Meter CR-400 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). Determinations were carried out on cross-sections of the sausage. Fifteen determinations were performed from each formulation.

The pH was determined using a pH meter (827pH Lab Methrom, Herisau, Switzerland) on 10 g homogenates in 100 ml of distilled water. Three measurements were performed per sample.

2.9. Microbiological analysis

Ten g of each sample (from 2 trays per sample) were taken and placed in a sterile plastic bag with 90 ml of peptone water (0.1%) with 0.85% NaCl. After 2 min. in a stomacher blender (Stomacher Colworth 400, Seward, UK), appropriate decimal dilutions were pour-plated (1 mL) on the following media: Plate Count Agar (PCA) (Merck, Germany) for the total viable count (TVC) (30 °C for 72 h); De Man, Rogosa, Sharp Agar (MRS) (Merck, Germany) for lactic acid bacteria (LAB) (30 °C for 3–5 days); and Violet Red Bile Glucose Agar (VRBG) (Merck, Germany) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of colony-forming units per gram (Log cfu/g).

2.10. Analysis of biogenic amines by ion-exchange chromatography

Tyramine, phenylethylamine, histamine, putrescine, cadaverine, tryptamine, agmatine, spermidine and spermine were determined in an extract prepared by blending 15 g of each sample with 30 mL of 7.5% trichloroacetic acid in an Ultraturrax homogenizer (IKA-Werke, Janke, & Kunkel, Staufen, Germany) (20,000 rpm, 3 min) and centrifuged at

5000 g for 15 min at 4 °C in a desktop centrifuge (Sorvall RTB6000B, DuPont, USA). The supernatants were filtered through a 0.45 μm Millipore filter, and 10 μL of this filtrate was injected into an HPLC model 1022 with a Pickering PCX 3100 post-column system (Pickering Laboratories, Mountain View, Ca, USA) following the methodology of Triki, Jiménez-Colmenero, Herrero, and Ruiz-Capillas (2012). The results are averages of at least 3 determinations.

2.11. Statistical analysis

One-way analyses of variance (ANOVA) to evaluate the statistical significance ($P<0.05$) of the effect of merguez sausage formulation and two-way ANOVA as a function of formulation and storage time were performed. Least squares differences were used for comparison of mean values among formulations and Tukey's HSD test to identify significant differences ($P<0.05$) between formulations and storage times. In addition, Pearson product moment correlation (r) determined the relationships between each parameter from the different analysis and storage time. The software used was SPSS 14.0 (SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. Proximate analysis, energy value and mineral content

As expected, partial (75%) and total (100%) replacement of beef fat with konjac materials (Table 1) affected ($P<0.05$) the proximate composition of the sausages (Table 2). Fat contents were affected ($P<0.05$) by formulation: levels were over 18% in control samples (C) and ranged between 4 and 12% in the reformulated merguez (Table 2), all in accordance with the product design. Compared with control samples, both partial and total replacement of beef fat by konjac gel (75/KG and 100/KG) resulted in products with less ($P<0.05$) fat content (8.6 and 4.3% respectively), making for a fat reduction of between 53% and 76% respectively. However, when beef fat was replaced in the same way by OKM, the fat reduction was less (34 and 49% respectively), which is consistent with the presence of olive oil in the konjac material and the proportion of animal fat replacement. This explains why 75/OKM sample had a higher ($P<0.05$) fat content than 100/OKM merguez (Table 2). In C, 75/KG and 100/KG formulations the total fat was of animal origin, whereas 75/OKM and 100/OKM merguez sausages contained around 4 and 6% olive oil, which means that approximately 23 and 36% respectively of the total fat in 75/OKM and 100/OKM, was olive oil (Table 2). Osburn and Keeton (1994) reported 30% fat reduction in pre-rigor fresh pork sausage using konjac gel as a fat replacer. Similarly, Lyons, Kerry, Morrissey, and Buckley (1998) formulated low-fat pork sausages (<3%) with 85% fat reduction by replacing (100%) of animal fat with a combination of whey protein/carrageenan gels and tapioca starch.

The protein content of the control sample was higher ($P<0.05$) than those of the reformulated sausages, with no differences ($P>0.05$) between them (Table 2). Since all formulations were prepared with the same meat content (Table 1), these differences must have been due mainly to the lower contribution of protein from the beef fat as a consequence of reformulation (Table 1). In general, as fat content decreased and konjac materials increased, moisture content ($P<0.05$) (Table 2) increased, ranging between 60.63% for controls and 76.96% for total replacement of beef fat with konjac gel (100/KG). The inverse relationship between fat and moisture contents is consistent with the reformulation (dilution effect): fat reduction was achieved by replacing the ingredient "beef fat" with konjac material containing more than 90% water. Quantitatively no relevant variations in ash content (2.43–2.57%) between formulations were observed (Table 2).

The energy content of the control sample (C) was 233 kcal/100 g (around 71% from fat), while in the reformulated ones it ranged from 99 to 171 kcal/100 g, with fat accounting for between 40% in 100/KG and 63% in 75/OKM (Table 2). In formulations with partial and total beef fat replacement by OKM (75/OKM and 100/OKM), olive oil accounted for 23 and 36% of the total energy content respectively. Similar energy reduction levels were reported by Osburn and Keeton (1994).

The minerals in meat products are important components for nutrition and health. The product reformulation altered the concentrations of some of the minerals in the merguez (Table 2). The calcium content of the control sample (C), as similarly reported in merguez (ANSES, 2008), was lower ($P<0.05$) than in the reformulated sausages (27.5–31.8 mg/100 g). Total beef fat replacement with konjac materials (100/KG and 100/OKM) produced sausages with higher ($P<0.05$) Ca concentrations than in sausages formulated with partial beef fat replacement (75/KM and 100/OKM). Since $Ca(OH)_2$ was used in the preparation of the konjac material (see Materials and methods section), the variation of Ca contents in the different formulations can be explained by the proportion of konjac materials used in each case. The magnesium contents of merguez (21–24 mg/100 g) were similar to the contents (21.1 mg/100 g) reported for merguez by ANSES (2008). In these products any reduction of the beef fat to improve fat content was accompanied by a reduction ($P<0.05$) in Mg content (Table 2). Meats contain an intermediate level of magnesium; however, 100 g of merguez can supply over 5% of the recommended daily amount (RDA 375 mg) (EC, 2008). This micronutrient is reported to have potential antiarrhythmic effects (Albert, Gaziano, Willett, & Manson, 2002).

Table 2
Proximate analysis (%), energy values (kcal/100 g) and mineral content (mg/100 g) of merguez sausages.

	C	75/KG	75/OKM	100/KG	100/OKM
Moisture	60.63 ± 1.07^{a}	72.16 ± 0.37^{c}	69.37 ± 0.08^{b}	76.96 ± 0.21^{d}	72.55 ± 0.56^{c}
Fat	18.41 ± 0.49^{d}	8.64 ± 0.57^{b}	12.01 ± 0.79^{c}	4.33 ± 0.02^{a}	9.28 ± 0.37^{b}
Protein	16.07 ± 0.53^{b}	13.98 ± 0.72^{a}	13.42 ± 0.31^{a}	12.42 ± 1.72^{a}	12.89 ± 0.69^{a}
Ashes	2.53 ± 0.05^{ab}	2.49 ± 0.02^{ab}	2.45 ± 0.04^{a}	2.57 ± 0.06^{b}	2.43 ± 0.04^{a}
Fat reduction (%)	–	53.0	34.8	76.5	49.6
Energy value	233.4	143.9	171.1	99.5	147.9
From total fat	167.5 (71.7)	78.6 (54.6)	108.1 (63.2)	39.4 (39.6)	84.5 (57.1)
From beef fat	167.5 (71.7)	78.6 (54.6)	68.5 (40.0)	39.4 (39.6)	31.7 (21.4)
From olive oil[a]	–	–	39.6 (23.2)	–	52.8 (35.7)
Calcium	19.25 ± 0.50^{a}	29.57 ± 1.09^{c}	27.49 ± 0.07^{b}	31.21 ± 0.68^{d}	31.84 ± 0.25^{d}
Magnesium	24.44 ± 0.70^{b}	21.68 ± 0.38^{a}	21.43 ± 0.37^{a}	20.92 ± 0.74^{a}	21.45 ± 0.44^{a}
Sodium	833.2 ± 47.18^{b}	759.7 ± 8.69^{a}	776.3 ± 18.04^{ab}	824.7 ± 0.06^{ab}	772.9 ± 25.15^{ab}
Potasium	289.5 ± 8.96^{b}	281.1 ± 2.65^{ab}	267.3 ± 6.35^{a}	293.1 ± 1.94^{b}	271.5 ± 6.71^{a}
Iron	1.68 ± 0.08^{b}	1.47 ± 0.04^{a}	1.78 ± 0.06^{b}	1.71 ± 0.06^{b}	1.37 ± 0.11^{a}

For sample denomination see Table 1. Energy content: calculation based on 9.1 kcal/g for fat and 4.1 kcal/g for protein and carbohydrates. In brackets, percentage of energy content. Means ± standard deviation. Different letters in the same row indicate significant differences ($P<0.05$).

[a] Calculated on the basis of formulation (20% OKM added is olive oil).

Formulation (Table 1) had no clear effect on sodium levels (Table 2), with values (760–833 mg/100 g) in the same range as reported for this product by ANSES (2008). However, considering the differences reported in the NaCl proportions (1.8–3.6%) used in merguez preparation (Benkerroum et al., 2003), sodium levels in these products may be expected to vary. The relationship between high salt (sodium) intake and high blood pressure is well known and is one of the three major risk factors for cardiovascular diseases (Antonios & Macgregor, 1997). No clear relationship was observed between potassium content (271–293 mg/100 g) and sample formulation (Table 2), with values much lower than reported by others for this product (ANSES, 2008). Merguez formulations contained iron levels ranging between 1.37 and 1.78 mg/100 g, which means that 100 g of the product would supply around 10% of the RDA (14 mg/day). Because meat is the predominant iron source, bioavailability is assured, and hence the new product can still have a major impact on groups vulnerable to iron deficiency, one of the most prevalent nutritional deficiencies, in both developing and developed nations (Neumann, Harris, & Rogers, 2002).

The difference between the mineral contents of merguez and other types of fresh sausage may additionally be related to the meat raw materials used (proportion and composition), and to the specific condiments used, some of which, like harissa, contain considerable amounts of minerals (66 mg/100 g Ca, 54 mg/100 g Mg, 818 mg/100 g Na 7.42 mg/100 g K and 2.1 mg/100 g Fe).

3.2. Sensory evaluation

Sensory evaluation of the different formulations was carried out at the outset of storage (Fig. 1). Some small but non-significant formulation-dependent differences were observed. Juiciness scores of products ranged between 4.78 and 5.71. Control samples received a similar ($P>0.05$) juiciness score to the rest of the sausages. Firmness scores for merguez were in the intermediate range, with no significant differences between sausages. General acceptability of merguez was likewise unaffected ($P>0.05$) by formulation, with all the products scoring well. There are no previous sensory studies on merguez sausage in the literature. However, in low-fat pre-rigor fresh pork sausage as the konjac gel content increased (0, 10 and 20%), sensory textural attributes came closer to those of the control (Osburn & Keeton, 1994). In breakfast sausages, overall acceptability decreased with fat content, so that it was higher in 13–29% fat products than in 5–9% fat content sausages, with minor changes observed in juiciness scores (Barbut & Mittal, 1995). No differences between high (19%) and reduced (13.5%) fat content were observed in firmness and juiciness of breakfast sausages (Morin, Temelli, & MacMullen, 2002), similar to the present results. The results of this study indicate that improving fat content did not negatively affect the sensory quality of the healthier merguez. However, it should be noted that the large amount of spices in these products can mask some changes associated with the formulation, so minor sensory variations may be missed.

3.3. Water and fat binding properties

To assess the effect of reformulation on the water and fat binding properties of the merguez sausages, these properties were estimated under different conditions, during processing (Table 3) and as affected by storage time (Table 3) and cooking treatment (Table 4). Processing losses, which ranged between 3.0 and 5.5%, were similar ($P>0.05$) in all sausages, except in the 75/KG sample which had the highest processing losses (Table 3). Purge losses (PL) were affected ($P<0.05$) by formulation and storage, with an interaction ($P<0.05$) between both factors (Table 3). PL increased over storage in all formulations, although the increase was only significant in the case of C and 75/KG. As with processing loss, the 75/KG sample generally had the highest PL values, which ranged between 2.77 and 5.04%. Purge loss is of interest because of its influence on the appearance (an important criterion for selection by consumers) and stability (it favors microbial growth) of these products, but there is very little information on fresh sausage-type products and none that the authors know of on merguez. Lyons et al. (1998) reported purge loss levels of around 5% in fresh pork sausage; however these data are not comparable with the present data since they refer to thawing loss (after 14 days at −20 °C).

Since these products are usually consumed after cooking, it is important to evaluate the ability of the system to bind water and fat after protein denaturation and aggregation and assess how well the moisture or juices are retained in the cooked product. Cooking loss (as well as water and fat losses) of different merguez formulations was affected ($P<0.05$) by the formulation and storage, with interaction ($P<0.05$) between both factors (Table 4). Although there were some variations in cooking loss as affected by formulation (Table 4), the results showed no clear relationship between this parameter (Table 4) and sausage composition (Table 2). While C sample was one of the sausages with the lowest cooking loss at the beginning of storage, at day 7 of storage it had the highest ($P<0.05$) cooking loss. Cooking loss increased ($P<0.05$) with storage time in all formulations; initial cooking loss values were 19–23%, whereas at the end of storage they were between 26 and 33%. Cooking loss proportions close to 18% have been reported in fresh pork sausages (Hayes, Stepanyan, Allen, O'Grady, & Kerry, 2011). Low-fat fresh pre-rigor pork sausages formulated with 20% konjac gel had higher cooking

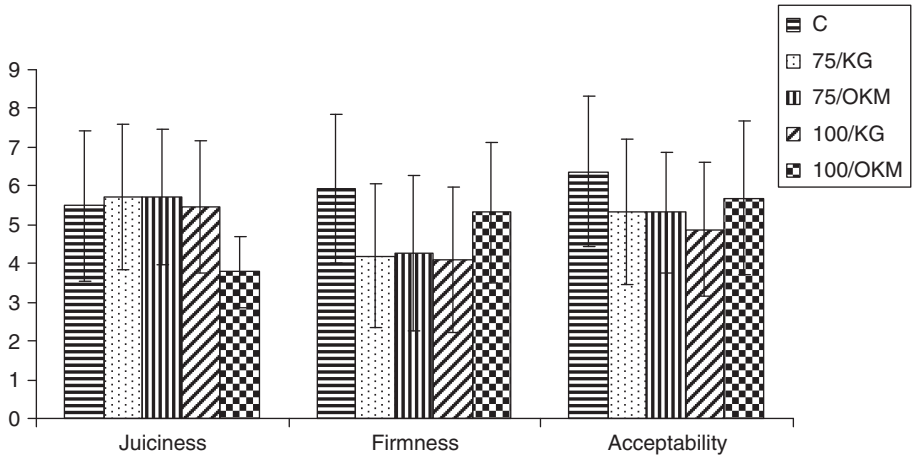

Fig. 1. Sensory evaluation of different merguez sausages during refrigerated storage. Standard deviations are represented by vertical intervals crossing the curve lines.

Table 3
Processing loss (%) and purge loss (%) of different merguez sausages.

Samples	Processing loss	Purge loss Storage time (days at 2 °C)		
		3	5	7
C	3.01 ± 0.48^{1}	0.98 ± 0.29^{a1}	1.47 ± 0.43^{b1}	2.09 ± 0.47^{b1}
75/KG	5.51 ± 0.61^{2}	2.77 ± 0.71^{a2}	3.87 ± 0.94^{ab2}	5.04 ± 1.48^{b2}
75/OKM	2.83 ± 0.55^{1}	0.90 ± 0.47^{a1}	1.72 ± 1.05^{a1}	2.38 ± 1.14^{a1}
100/KG	3.86 ± 0.77^{1}	1.22 ± 0.55^{a1}	1.29 ± 0.60^{a1}	2.65 ± 0.62^{a1}
100/OKM	3.12 ± 0.38^{1}	1.35 ± 0.49^{a12}	2.20 ± 0.44^{a12}	2.95 ± 0.86^{a1}

For sample denomination see Table 1. Means ± Standard deviation. Different letters in the same row and different numbers in the same column are significantly different ($P<0.05$).

losses (around 24%) than those prepared with 0 and 10% konjac gel (close to 28%) (Osburn & Keeton, 1994). Cooking losses around 8% were reported in fresh pork sausage in which fat was replaced with whey protein/carrageenan gels and tapioca starch (Lyons et al., 1998). In low-fat fresh pork sausages at lower fat concentration (15 and 25%), cooking losses increased (16-30%) as added water increased (Ahmed, Miller, Lyon, Vaughters, & Reagan, 1990). However, small differences in cooking losses (27–29%) as affected by fat content (5–29%) were found in breakfast sausages (Barbut & Mittal, 1995). No references were found to the effect of storage time on cooking loss of this type of fresh sausages.

A number of interesting points emerged when the water and fat lost in cooking were quantified. Generally, water loss behaved similarly to cooking loss (Table 4). Its behavior could not be related to the moisture content of the formulations (Table 2), possibly because the water in the konjac materials (around 90% of their composition) was well bound and so had little bearing on the water cooking loss in the meat system. A similar effect has been reported in connection with the presence of konjac in cooked meat products (Jiménez-Colmenero et al., 2010). Water loss increased ($P<0.05$) with storage time in all formulations, although this effect was more pronounced in C sample, which had the lowest ($P<0.05$) initial water loss, while at the end of storage all formulations registered similar ($P>0.05$) water losses (Table 4). In C sample water loss over storage was around 67–75% (of cooking loss) as compared to over 84% in the other formulations (Table 4). The highest ($P<0.05$) fat cooking loss over storage was observed in the control sample. In general, fat loss was influenced more by the concentration than by the type of fat, since fat loss increased ($P<0.05$) with storage time only in the cases of C and 75/OKM formulations, which were the ones with the highest fat contents: 18.4% and 12.0% respectively (Table 2). In merguez with less than 10% fat (Table 2), fat loss did not change ($P>0.05$) over storage irrespective of the relative proportion of olive oil/animal fat in the formulation. In control samples the fat loss accounted for between 25 and 32% of cooking loss, as compared to less than 16% in the other formulations (Table 4). In fact a high (between 0.756 and 0.938) and significant ($P<0.001$) correlation was observed between product fat content and fat cooking loss during the storage.

Table 4
Cooking loss (%) of different merguez sausages during refrigerated storage.

	Storage time (days at 2 °C)		
	3	5	7
Cooking loss (%)			
C	18.88 ± 0.56^{a1}	28.05 ± 1.47^{b3}	33.05 ± 0.23^{c2}
75/KG	23.20 ± 0.91^{a3}	25.90 ± 0.43^{b2}	27.18 ± 0.30^{b1}
75/OKM	22.67 ± 0.94^{a3}	27.51 ± 0.35^{b23}	27.63 ± 1.22^{b1}
100/KG	19.73 ± 0.30^{a12}	23.19 ± 0.11^{b1}	27.20 ± 1.67^{c1}
100/OKM	21.42 ± 0.53^{a23}	23.62 ± 0.55^{b1}	25.98 ± 1.97^{c1}
Water loss (%)			
C	14.10 ± 0.37^{a1}	19.07 ± 1.76^{b1}	23.17 ± 0.66^{c1}
75/KG	19.51 ± 1.58^{a2}	22.89 ± 0.07^{b2}	24.38 ± 0.25^{b1}
75/OKM	19.78 ± 1.57^{a2}	23.31 ± 0.90^{b2}	23.35 ± 0.71^{b1}
100/KG	18.23 ± 0.30^{a2}	21.46 ± 0.07^{b12}	25.25 ± 1.09^{c1}
100/OKM	19.40 ± 0.48^{a2}	21.48 ± 0.57^{ab2}	23.40 ± 1.99^{b1}
Fat loss (%)			
C	4.78 ± 0.30^{a4}	8.97 ± 0.35^{b4}	9.88 ± 0.77^{b3}
75/KG	3.69 ± 0.87^{a3}	3.00 ± 0.45^{a2}	2.80 ± 0.07^{a1}
75/OKM	2.43 ± 0.16^{a2}	4.20 ± 0.80^{b3}	4.28 ± 0.51^{b2}
100/KG	1.49 ± 0.02^{a1}	1.74 ± 0.06^{a1}	1.95 ± 0.59^{a1}
100/OKM	2.02 ± 0.12^{a12}	2.13 ± 0.05^{a12}	2.58 ± 0.18^{a1}

For sample denomination see Table 1. Means ± Standard deviation. Different letters in the same row and different numbers in the same column are significantly different ($P<0.05$).

3.4. Lipid oxidation (TBARS)

TBARS values of the different merguez formulations were affected ($P<0.05$) by the formulation and storage (Table 5), with interaction ($P<0.05$) between both factors. Throughout the experiment there was generally no appreciable correlation ($P>0.05$) between TBARS levels (Table 5) and fat content (Table 2). Lipid oxidation increased ($P<0.05$) during storage from initial TBARS values of 0.28 and 0.41 mg MDA/kg to 0.50–0.97 mg MDA/kg at the end of storage. The formulations with olive oil incorporated (75/OKM and 100/OKM) registered the highest levels of TBARS (0.71–0.97 mg/MDA/kg respectively) (Table 5). The presence of olive oil should favor the presence of MUFAs and hence the level of lipid unsaturation, but they have also been reported to contain antioxidants that would limit the oxidation process. In fact no oxidation problems have been detected in partial substitution of pork backfat by olive oil in cooked and fermented meat products (Jiménez-Colmenero, 2007); replacing beef fat with olive oil has been reported to favor lipid oxidation in traditional Turkish dry fermented sausage (Kayaardi & Gök, 2003), but this effect was not observed using olive oil (0.1–1.1%) in fresh sausage (Serrano-Perez, 2005). TBARS contents lower than 0.09 mg/kg were found after refrigerated storage (14 days) of Italian fresh sausages (Kamdem, Francesca, & Guerzoni, 2007). In the present experiment the TBARS values, even at the end of storage, were lower than 1 mg/kg (Table 5). It has been reported that a meat sample containing TBARS levels from 0.5 to 1 possessed no off odors (Tarladgis, Watts, Younathan, & Dugan, 1960) and that between 1 and 2 mg/kg of malonaldehyde is the minimum detectable level for oxidized flavor in beef (Watts, 1962) and its products for an inexperienced panel (Greene & Cumuze, 1981). Values below 1.36 mg MDA/kg do not promote off-flavors detectable by trained sensory evaluation in processed meat products (Liu, Kerry, & Kerry, 2006). The relatively low level of lipid oxidation in merguez can be related to various factors; its short shelf life, limitation of the substrate available for lipid oxidation due to fat reduction, and the presence of antioxidants in some of the spices used (Kamdem et al., 2007).

Table 5
Lipid oxidation as changes in thiobarbituric acid-reactive substances (TBARS mg MDA/kg sample) values of merguez sausages during refrigerated storage.

Samples	Storage time (days at 2 °C)			
	0	3	5	7
C	0.37 ± 0.01^{a3}	0.40 ± 0.01^{ab3}	0.43 ± 0.02^{b2}	0.57 ± 0.00^{c2}
75/KG	0.32 ± 0.01^{a2}	0.35 ± 0.01^{a2}	0.47 ± 0.06^{b3}	0.50 ± 0.01^{b1}
75/OKM	0.28 ± 0.02^{a1}	0.29 ± 0.02^{a1}	0.36 ± 0.04^{b1}	0.71 ± 0.04^{c3}
100/KG	0.31 ± 0.02^{a12}	0.45 ± 0.01^{b4}	0.47 ± 0.00^{b23}	0.51 ± 0.01^{c1}
100/OKM	0.41 ± 0.01^{a4}	0.42 ± 0.04^{a34}	0.52 ± 0.02^{b4}	0.97 ± 0.02^{c4}

For sample denomination see Table 1. Means ± Standard deviation. Different letters in the same row and different numbers in the same column are significantly different ($P<0.05$).

3.5. Color

Meat product color is a primary determinant of appearance, and thus influences consumers' decisions to buy. Color parameters of different merguez formulations were affected ($P<0.05$) by the formulation and storage, with interaction ($P<0.05$) between both factors (Table 6). The effect of fat reduction by replacement of beef fat with KG (comparing C versus 75/KG and 100/KG) generally reduced ($P<0.05$) L* and b* values. There was a significant decrease in lightness levels in controls at the end of storage, while in the case of 100/KG formulations there was an increase ($P<0.05$) after 7 days of storage (Table 6). No obvious data trends related to formulation were observed in yellowness of stored merguez. The presence of olive oil in the formulation reduced the differences induced in these color parameters by fat reduction. Similarly, fat reduction generally reduced ($P<0.05$) the a* value (Table 6). And again, there was a very pronounced decrease in redness ($P<0.05$) during storage in all merguez types.

The addition of konjac gel to low-fat pre-rigor fresh pork sausages tended to reduce L* and increase a* when compared to control fat products, while redness decreased and lightness increased with storage time (Osburn & Keeton, 1994). In low-fat fresh pork sausages increasing the fat percentage resulted in increased lightness and yellowness and reduced redness (Ahmed et al., 1990). However, in breakfast sausages lightness and yellowness were found to be directly proportional to the fat level, while minor changes were observed in redness (Barbut & Mittal, 1995). Boles, Mikkelsen, and Swan (1998) showed that redness of fresh beef sausage decreased with storage time and that these sausages also became darker and less yellow. As in the present study, Hayes et al. (2011) reported decreased redness in raw pork sausages stored at 4 °C, although the behavior of lightness during storage was conditioned by the product formulation. In any case, for the purposes of the present study it should be noted that since merguez is a strongly-spiced meat product, these compounds may influence the effect of the variables considered on color.

3.6. pH

The pH of the merguez formulations was affected ($P<0.05$) by the formulation and storage, with interaction ($P<0.05$) between both factors (Fig. 2). The initial pH ranged from 5.81 to 5.88; although the control sample had the lowest ($P<0.05$) pH, the formulation-dependent variations observed were quantitatively small. The pH decreased ($P<0.05$) with storage time in all formulations, and the highest ($P<0.05$) pH values, from day 5 of storage were registered by the control. At the end of storage, the formulations with the higher konjac concentration (100/KG and 100/OKM) had lower ($P<0.05$) pH values (Fig. 2). Changes in pH over storage may be related to microbial growth. The behavior of pH in the different formulation could be due to the fact that lactic acid bacteria can ferment carbohydrates like Konjac gel, and the pre-gelatinized starch used in the formulation can likely serve as a source of fermentable carbohydrate. This would explain why the pH of the lots containing KG was lower than in the control samples, while they contained the same level of micro-organisms (Table 7). Moreover, a decrease in pH can be caused by an increase of lactic acid content, especially *Lactobacillus*, which is often associated with fresh meat (Holmer, McKeith, & Killefer, 2008; Salazar, García, & Selgas, 2009). In merguez sausage, Benkerroum et al. (2003) observed that pH decreased to a mean value of 5.4 at the end of the storage period (3 to 5 days), suggesting that the raw material contained naturally-acidifying bacteria responsible for spontaneous acidification of merguez sausage. Hayes et al. (2011) observed a significant decline in pH levels in raw pork sausages stored in MAP (modified atmosphere) at 4 °C, from 6.4 at day 0 to 5.0 at day 21 of storage. Since pH affects the water binding properties of meat systems, and lower pH means more water loss from the preparation (Huff-Lonergan & Lonergan, 2005), the decrease of pH with storage time may help to explain the changes in weight loss in merguez sausages (Tables 4 and 5).

3.7. Microbiology

The microbial counts of the different formulations were affected ($P<0.05$) by the formulation and storage, with interaction ($P<0.05$) between both factors (Table 7). The initial levels of total aerobic microorganisms (TVC), lactic acid bacteria (LAB) and enterobacteria counts were 5.64–5.97, 5.34–5.40 log cfu/g and 3.44–3.83 Log cfu/g respectively. The levels of TCV in these formulations were below the limit of 6 log cfu/g, which is the acceptable total microbial quality standard for fresh sausages. The TVC and *enterobacteriaceae* were higher ($P<0.05$) in control sausage, whereas lactic bacteria counts did not differ significantly between formulations. At day 3 of storage, a significant ($P<0.05$) increase was observed in the microbial population, which reached levels in excess of 7 and 6 Log cfu/g in the TVC and LAB respectively (Table 7). This increase was associated with a corresponding rapid decrease in pH (Table 2) due to the metabolic activity of these bacteria. Similar behavior of some lactic acid bacteria has been reported in studies of fresh beef, pork and lamb during chill storage (Egan, Eustace, & Shay, 1988; Ruiz-Capillas, Cofrades, Serrano, & Jiménez-Colmenero, 2004).

Microorganism levels increased slightly up to day 5, after which there were no significant changes up to the end of storage. At the end of storage the highest ($P<0.05$) levels (TVC, LAB and *enterobacteriaceae*) were registered in the controls, where LAB was the dominant flora

Table 6
Colour parameters (lightness, L*; redness, a*; yellowness, b*) of different merguez sausages during refrigerated storage.

Parameters	Samples	Storage time (days at 2 °C)			
		0	3	5	7
L*	C	51.14 ± 0.94^{b3}	50.86 ± 0.89^{ab3}	52.15 ± 1.23^{b4}	49.74 ± 1.39^{a3}
	75/KG	46.06 ± 0.61^{a2}	47.36 ± 1.02^{ab2}	47.91 ± 0.59^{b3}	46.02 ± 1.11^{a12}
	75/OKM	46.74 ± 1.37^{a2}	46.17 ± 0.67^{a2}	46.97 ± 1.39^{a23}	46.93 ± 1.33^{a2}
	100/KG	43.51 ± 1.17^{a1}	44.72 ± 1.25^{ab1}	43.65 ± 0.90^{a1}	45.52 ± 0.80^{b1}
	100/OKM	45.46 ± 1.29^{a2}	46.12 ± 1.25^{ab2}	46.12 ± 1.07^{ab2}	46.97 ± 0.66^{b2}
a*	C	16.41 ± 0.89^{c2}	15.81 ± 0.91^{c2}	13.83 ± 1.04^{b3}	11.77 ± 0.67^{a3}
	75/KG	16.34 ± 1.03^{d2}	14.55 ± 1.06^{c1}	11.57 ± 0.60^{b12}	8.70 ± 0.40^{a1}
	75/OKM	16.11 ± 1.43^{d12}	13.73 ± 0.74^{c1}	12.28 ± 0.89^{b2}	10.05 ± 0.65^{a2}
	100/KG	15.17 ± 1.16^{d1}	14.06 ± 0.63^{c1}	10.77 ± 0.54^{b1}	8.55 ± 0.57^{a1}
	100/OKM	16.55 ± 0.88^{d2}	14.75 ± 0.48^{c12}	11.73 ± 0.72^{b12}	8.96 ± 1.01^{a1}
b*	C	17.22 ± 0.95^{a3}	20.42 ± 1.30^{b4}	21.38 ± 1.18^{bc3}	22.76 ± 1.69^{c4}
	75/KG	13.66 ± 1.02^{a2}	15.87 ± 2.29^{b3}	16.90 ± 0.95^{b2}	13.67 ± 1.22^{a12}
	75/OKM	13.71 ± 2.26^{a2}	12.76 ± 1.81^{a2}	17.16 ± 1.14^{b2}	15.75 ± 1.09^{b3}
	100/KG	9.89 ± 2.45^{a1}	10.88 ± 1.29^{ab1}	14.07 ± 0.84^{c1}	12.47 ± 1.55^{bc1}
	100/OKM	13.66 ± 1.69^{a2}	14.81 ± 1.39^{a3}	20.04 ± 1.27^{b3}	15.37 ± 1.28^{a23}

For sample denomination see Table 1. Means ± Standard deviation. Different letters in the same row and different numbers in the same column are significantly different ($P<0.05$).

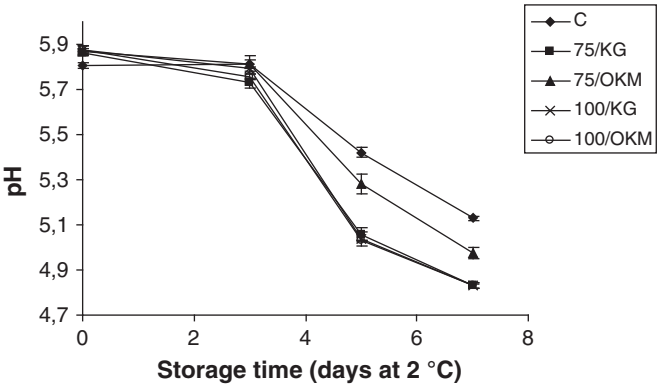

Fig. 2. pH levels of different merguez sausages during refrigerated storage. Standard deviations are represented by vertical intervals crossing the curve lines.

(Table 7). Dominance of LAB in the spoilage flora of these meat products has also been reported by Korkeala, Alanko, Makeli, and Lindroth (1989). The lower growth rate of the microbiota in this second phase may be attributed to the low temperature (2 °C). Sachindra, Sakhare, Yashoda, and Narasimha Rao (2005) showed that in refrigerated storage (4 °C) buffalo sausage total plate counts increased by two logarithmic units after 16 days and LAB counts doubled. Hayes et al. (2011) observed a large increase of TVC from 3.24 to 5.68 Log cfu/g after 7 days storage at 2 °C in raw pork sausages.

3.8. Biogenic amines

The BA contents of the different merguez formulations were affected ($P<0.05$) by the formulation and storage, with interaction ($P<0.05$) between both factors (Table 8). The physiological amines spermidine and spermine registered the highest concentrations, at 1.50–2.10 mg/kg and 11.23–15.64 mg/kg respectively. Levels of these amines were lowest ($P<0.05$) in the merguez containing the higher KG level (100/KG). There were no significant differences among the other formulations. Spermidine levels were similar to those reported in other studies on restructured beef products, but spermine levels were lower (Ruiz-Capillas et al., 2004). This could be due mainly to the spermine content in the meat raw material and to formulation conditions (beef content). The levels of these amines in beef and beef products generally vary (Ruiz-Capillas & Jiménez-Colmenero, 2004); indeed, other authors have reported no spermine in beef (Smith, Kenney, Kastner, & Moore, 1993). Initial levels of the other biogenic amines were very low (<2 mg/kg) (Table 8); 0.20 mg/kg of agmatine was registered (data not shown) and no tryptamine was detected. Such values are common in fresh sausages, although they tend to vary widely in products of this kind (Ruiz-Capillas et al., 2004). There were changes in spermidine and spermine levels during storage; these were significant in some cases but were generally of little practical importance. Levels of all the other BAs increased ($P<0.05$); in the case of the typical spoilage amines putrescine and cadaverine they were low, with putrescine concentrations<3 mg/kg and cadaverine levels even lower (Table 8). Tyramine behaved differently: by day 3 of storage it had reached between 12.95 and 24.52 mg/kg (with lower values in 75/OKM and 100/KG), and levels had reached between 32.64 and 42.01 mg/kg by the end of storage. Tyramine was the amine at the highest concentrations. Lower concentrations of tyramine have been reported in beef products (Ruiz-Capillas et al., 2004), possibly due to differences in the type of microbial flora, which may be influenced by the large amount of ingredients used in these products. Many of these ingredients have been reported to act as antimicrobial agents (Kamdem et al., 2007) with the ability to act on the microbial flora capable of growing in these conditions. As several authors have reported (Ruiz-Capillas, Jiménez-Colmenero, Carrascosa, & Muñoz, 2007), there is some strain specificity so that strains with different biogenic amine-producing capacities may grow selectively at the same level of mircoorganism counts. In the case of tyramine, levels of which are related to the growth of lactic acid bacteria (de las Rivas et al., 2008), some authors have found, for example, that growth of *L. sakei*, does not produce biogenic amines (Roig-Sagués & Eerola, 1997). This could also explain why high histamine concentrations were found at the end of storage and these were higher in the reformulated sausages, especially 75/KG (20.32 mg/kg) sample. Although this amine is not typical of fresh meat products (Ruiz-Capillas & Jiménez-Colmenero, 2004), similar concentrations to those found in the present study have been reported in fresh beef products (Ruiz-Capillas et al., 2004). Generally speaking, except for the case of histamine, the results do not show a clear connection between the formation of biogenic amines and fat improving strategy, either in formulations where beef fat was replaced by konjac gel (reduced fat content), or where olive oil stabilized in a konjac matrix was added (fat reduction plus addition of healthier oil combination).

Some authors have reported that 700–800 mg/kg (ten Brink, Damink, Joosten, & Huis in´t Veld, 1990), or even 125 mg/kg (Vidal-Carou, Izquierdo, Matín-Morro, & Marine-Font, 1990) of tyramine is enough to be toxic in a normal person, and in the case of histamine the Food and Drug Administration has set a 50 mg/kg concentration as the safe permitted limit (FDA, 2001). However, other amines such as putrescine and cadaverine are also implicated, as they increase histamine toxicity (FDA, 2001). The levels found in this study are clearly well below those defined as toxic, and therefore in that

Table 7
Microbiological counts (Log cfu/g) in different merguez sausages during refrigerated storage.

Microorganisms	Samples	Storage time (days at 2 °C)			
		0	3	5	7
Total viable count	C	5.97 ± 0.04^{a3}	8.00 ± 0.00^{b3}	8.39 ± 0.01^{c4}	8.51 ± 0.07^{c4}
	75/KG	5.78 ± 0.02^{a2}	7.31 ± 0.05^{b2}	7.84 ± 0.04^{d2}	7.62 ± 0.10^{c2}
	75/OKM	5.71 ± 0.03^{a12}	7.29 ± 0.02^{b2}	8.06 ± 0.03^{c3}	8.08 ± 0.05^{c3}
	100/KG	5.64 ± 0.09^{a1}	6.97 ± 0.09^{b1}	7.41 ± 0.02^{c1}	7.37 ± 0.04^{c1}
	100/OKM	5.65 ± 0.03^{a1}	7.02 ± 0.13^{b1}	7.45 ± 0.13^{c1}	7.40 ± 0.05^{c1}
Lactic acid bacteria	C	5.37 ± 0.07^{a1}	7.02 ± 0.04^{c3}	7.17 ± 0.12^{d4}	6.72 ± 0.06^{b4}
	75/KG	5.35 ± 0.04^{a1}	6.50 ± 0.10^{c1}	6.89 ± 0.13^{d1}	6.35 ± 0.10^{b1}
	75/OKM	5.40 ± 0.05^{a1}	6.72 ± 0.01^{b2}	7.10 ± 0.02^{c34}	6.61 ± 0.06^{b3}
	100/KG	5.34 ± 0.00^{a1}	6.70 ± 0.04^{c2}	7.04 ± 0.06^{c23}	6.25 ± 0.03^{b1}
	100/OKM	5.36 ± 0.03^{a1}	6.54 ± 0.06^{b1}	6.95 ± 0.06^{c12}	6.49 ± 0.06^{b2}
Enterobacteriaceae	C	3.83 ± 0.01^{a3}	4.72 ± 0.03^{bc2}	4.80 ± 0.09^{c4}	4.62 ± 0.06^{b4}
	75/KG	3.44 ± 0.06^{b1}	3.66 ± 0.00^{c2}	3.38 ± 0.11^{b1}	3.15 ± 0.15^{a1}
	75/OKM	3.63 ± 0.01^{b2}	3.46 ± 0.02^{a1}	3.82 ± 0.01^{c3}	3.76 ± 0.06^{bc3}
	100/KG	3.48 ± 0.05^{b12}	3.42 ± 0.16^{b1}	3.55 ± 0.17^{b2}	3.20 ± 0.12^{a1}
	100/OKM	3.54 ± 0.04^{b12}	3.35 ± 0.21^{a1}	3.64 ± 0.13^{b2}	3.52 ± 0.04^{b2}

For sample denomination see Table 1. Means ± Standard deviation. Different letters in the same row and different numbers in the same column are significantly different ($P<0.05$).

Table 8
Biogenic amines levels (mg/kg) in different merguez sausages during refrigerated storage.

Biogenic amines	Samples	Storage time (days at 2 °C)			
		0	3	5	7
Tyramine	C	0.69 ± 0.02^{a1}	21.99 ± 0.06^{b4}	26.21 ± 0.12^{c1}	33.84 ± 0.32^{d2}
	75/KG	2.06 ± 0.02^{a3}	19.14 ± 0.04^{b3}	33.84 ± 0.57^{c5}	41.46 ± 0.12^{d4}
	75/OKM	0.77 ± 0.01^{a12}	12.95 ± 0.00^{b1}	31.85 ± 0.74^{c4}	42.01 ± 0.53^{d4}
	100/KG	1.24 ± 0.02^{a123}	14.86 ± 0.01^{b2}	29.45 ± 0.22^{c3}	38.42 ± 0.11^{d3}
	100/OKM	1.61 ± 0.18^{a23}	24.52 ± 0.06^{b5}	27.91 ± 0.36^{c2}	32.64 ± 0.40^{d1}
Histamine	C	1.09 ± 0.17^{a1}	1.26 ± 0.05^{a1}	1.51 ± 0.45^{a1}	6.29 ± 0.94^{b1}
	75/KG	0.92 ± 0.06^{a1}	1.42 ± 0.00^{a1}	3.37 ± 0.41^{b2}	20.32 ± 0.07^{c5}
	75/OKM	1.09 ± 0.04^{a1}	1.79 ± 0.02^{a1}	2.38 ± 0.02^{a12}	10.75 ± 0.70^{b2}
	100/KG	0.96 ± 0.15^{a1}	1.41 ± 0.02^{ab1}	2.54 ± 0.20^{b12}	14.80 ± 0.00^{c4}
	100/OKM	0.93 ± 0.01^{a1}	1.57 ± 0.00^{b1}	2.26 ± 0.12^{c12}	12.31 ± 0.39^{d3}
Phenylethylamine	C	ND	0.31 ± 0.03^{a2}	0.74 ± 0.06^{b2}	1.16 ± 0.03^{c1}
	75/KG	0.21 ± 0.01^{a}	0.53 ± 0.01^{b3}	1.12 ± 0.09^{c3}	1.67 ± 0.07^{d2}
	75/OKM	ND	0.13 ± 0.01^{a1}	0.49 ± 0.03^{b1}	1.08 ± 0.05^{c1}
	100/KG	ND	0.10 ± 0.01^{a1}	1.23 ± 0.05^{b4}	1.69 ± 0.01^{c2}
	100/OKM	ND	0.04 ± 0.00^{a1}	0.40 ± 0.02^{b1}	1.08 ± 0.09^{c1}
Putrescine	C	0.67 ± 0.06^{a1}	0.90 ± 0.03^{a1}	1.56 ± 0.17^{b1}	1.99 ± 0.06^{c1}
	75/KG	1.54 ± 0.20^{a3}	1.59 ± 0.01^{a2}	1.99 ± 0.19^{b2}	2.44 ± 0.02^{c2}
	75/OKM	0.77 ± 0.07^{a1}	1.39 ± 0.00^{b2}	1.65 ± 0.20^{b1}	2.62 ± 0.03^{c2}
	100/KG	1.18 ± 0.14^{a2}	1.48 ± 0.03^{ab2}	1.68 ± 0.26^{b12}	2.31 ± 0.03^{c2}
	100/OKM	1.11 ± 0.04^{a2}	1.33 ± 0.01^{ab2}	1.60 ± 0.04^{bc1}	1.79 ± 0.03^{c1}
Cadaverine	C	0.04 ± 0.01^{a1}	0.08 ± 0.00^{b2}	0.36 ± 0.01^{c3}	1.16 ± 0.04^{d5}
	75/KG	ND	ND	0.04 ± 0.01^{a1}	0.23 ± 0.00^{b4}
	75/OKM	0.03 ± 0.00^{a1}	0.03 ± 0.00^{a1}	0.06 ± 0.01^{b2}	0.20 ± 0.00^{c3}
	100/KG	ND	ND	0.05 ± 0.00^{a12}	0.10 ± 0.00^{b2}
	100/OKM	ND	0.03 ± 0.00^{a1}	0.05 ± 0.00^{b12}	0.08 ± 0.00^{c1}
Spermidine	C	1.94 ± 0.01^{a2}	1.59 ± 0.05^{a1}	1.56 ± 0.06^{a1}	1.58 ± 0.22^{a12}
	75/KG	2.10 ± 0.33^{a2}	2.08 ± 0.09^{a2}	1.91 ± 0.45^{a1}	2.21 ± 0.08^{a3}
	75/OKM	1.97 ± 0.22^{b2}	1.56 ± 0.01^{ab1}	1.49 ± 0.33^{a1}	1.79 ± 0.11^{ab2}
	100/KG	1.50 ± 0.09^{a1}	1.51 ± 0.05^{a1}	1.54 ± 0.38^{a1}	1.67 ± 0.06^{a12}
	100/OKM	2.05 ± 0.15^{b2}	1.61 ± 0.00^{ab1}	1.84 ± 0.03^{b1}	1.32 ± 0.02^{a1}
Spermine	C	15.42 ± 0.27^{a2}	14.10 ± 0.32^{a2}	14.76 ± 1.37^{a2}	16.24 ± 2.27^{a2}
	75/KG	14.01 ± 1.19^{a2}	14.25 ± 0.20^{a2}	14.19 ± 1.65^{a2}	17.47 ± 0.30^{b2}
	75/OKM	15.29 ± 1.16^{bc2}	12.44 ± 0.10^{a12}	13.27 ± 1.23^{ab12}	16.75 ± 0.43^{c2}
	100/KG	11.23 ± 0.83^{a1}	10.46 ± 0.05^{a1}	12.10 ± 0.21^{ab1}	14.06 ± 0.16^{b1}
	100/OKM	15.64 ± 1.39^{b2}	14.00 ± 0.07^{ab2}	13.94 ± 0.14^{ab12}	12.29 ± 0.28^{a1}

For sample denomination see Table 1. Means ± Standard deviation. Different letters in the same row and different numbers in the same column are significantly different ($P<0.05$).
ND: Not detected

respect they pose no risk for consumers. In any case it is worth noting that organisms possess detoxifying mechanisms, and in normal circumstances the human body is able to quickly detoxify the histamine and tyramine absorbed from foods by means of the enzymes monoamine oxidase (MAO; EC 1.4.3.4), diamine oxidase (DAO; EC 1.4.3.6), and polyamine oxidase (PAO; EC 1.5.3.11) (Bardócz, 1995). However, the toxicity of these amines in the body depends on the efficiency of the body's detoxification system (Bardócz, 1995).

4. Conclusion

The reformulation process with konjac gel and olive oil stabilized in a konjac matrix opens up new possibilities for fat reduction and improvement of fatty acid profiles in North African fresh sausage (merguez). In this study replacement of beef fat by konjac gel reduced the fat content of merguez sausage by up to 76%. On the other hand, when olive oil in konjac was used as the fat replacement, fat reduction levels were lower (34–49%), although in these cases around 23 and 36% of the total fat was olive oil. Merguez contains considerable amounts of some minerals: for instance, 100 g supplies over 5% of the RDA of magnesium and around 10% of the RDA of iron. Also, improving fat content did not negatively affect the sensory quality of the healthier merguez, which had a relatively low level of lipid oxidation. It was not possible to establish a clear connection between biogenic amine formation and fat improvement strategy during refrigerated storage, but in any case these compounds do not pose any risk to consumers. The shelf life of merguez sausage was not affected by formulation. Therefore, this processing strategy is suitable for use in the development of healthier merguez sausages.

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VI.3.2. Storage stability of low-fat sodium reduced fresh merguez sausage prepared with olive oil in konjac gel matrix.

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Storage stability of low-fat sodium reduced fresh merguez sausage prepared with olive oil in konjac gel matrix

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ABSTRACT

This paper evaluates the nutritional values and stability during refrigerated storage of fresh beef merguez sausage as affected by a reformulation process which modified the fat content both by reducing fat (replacing beef fat with konjac gel) and incorporating olive oil (replacing beef fat with olive oil stabilized in a konjac matrix) and by reducing sodium content, replacing sodium chloride with a salt mixture (containing potassium chloride, calcium chloride and magnesium chloride). A preservative (sodium metabisulphite) was also used to extend the shelf-life of the product. The fat was reduced by 32 to 80% and sodium by over 36%. The reformulation did not negatively affect the sensory evaluation. Low microbiota growth rate and biogenic amines were attributed mainly to the presence of sodium metabisulphite. This preservative could be used in the reformulation to enhance safety and/or extend the shelf-life of this type of product.

1. Introduction

Merguez is a North African type of fresh sausage made with either beef or lamb or both and is widely consumed worldwide, including in Europe. Merguez has a short shelf-life even when stored at refrigerated temperatures (Benkerroum, Daoudi, & Kamal, 2003; Triki, Herrero, Jiménez-Colmenero, & Ruiz-Capillas, 2013). This sausage normally contains relatively high amounts of fats (over 20%), with a sodium content around 800 mg/100 g (ANSES, 2008; Triki et al., 2013). To produce healthier merguez sausage, the fat content needs to be modified or reduced and the sodium level also reduced. There is increasing interest among consumers and producers in reducing the use of sodium in meat processing (Desmond, 2006).

Modifying the fat content of meat-based foods by reducing the fat content and/or improving the fatty acid profile by replacing the animal fat normally present with a plant-based alternative is an important strategy for improving human health in many countries, including in the Maghreb and N. Africa. Konjac glucomannan (E-425) has been used as a fat analogue in various meat products including fresh pork sausages and merguez sausages (Osburn & Keeton, 1994; Triki et al., 2013). Triki et al. (2013) formulated merguez sausages replacing beef fat with konjac gel and olive oil stabilized in a konjac matrix reducing the fat content of merguez sausage by up to 76%. On the other hand, when olive oil was stabilized in a konjac matrix lower fat reduction levels (34–49%) were obtained, although in these cases around 23% and 36% of the total fat was olive oil. As a result, although this processing strategy can be considered as suitable for use in the development of healthier merguez sausages (Triki et al., 2013), the products still had two main drawbacks: the high sodium content (700–800 mg/100 g) and the limited shelf-life (less than 5 days at 2 °C), which is an important commercial consideration.

Excessive salt intake is a major risk for certain sectors of the population prone to increased blood pressure and therefore a risk of serious health problems including cardiovascular disease, diabetes, and kidney disease (Desmond, 2006; Toldrá & Reig, 2011). There are several strategies for reducing sodium in processed meat, with one of the most common being the replacement of all or part of the NaCl with other chloride salts, usually combinations of sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$). This approach has been widely used for different meat products (Desmond, 2006; Ruusunen et al., 2005; Tobin, O'Sullivan, Hamill, & Kerry, 2012; Toldrá & Reig, 2011; Zanardi, Ghidini, Conter, & Ianieri, 2010) with only very limited applications of this approach to fresh sausages (Pasin et al., 1989).

Strategies to increase the shelf-life of meat products include the use of preservatives such as organic acid, nitrite and bacteriocins and especially sulphites (Ruiz-Capillas & Jiménez-Colmenero, 2009). Their primary function is as a preservative or antioxidant to prevent or reduce spoilage (Ruiz-Capillas & Jiménez-Colmenero, 2009). Sulphites have been used in different meat products, including some with similar characteristics to merguez sausages such as burger meat or breakfast sausages and some fresh sausages such as fresh *longaniza* and *butifarra* (Directive, 2006/52/EC).

There are no references in the literature to studies combining reformulation processes based on fat modification and sodium reduction

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strategies associated with increased shelf-life in products like fresh merguez sausage. The aim of this paper is to evaluate the nutritional consequences, quality characteristics and refrigerated storage stability (shelf-life) of fresh beef merguez sausage after a reformulation process which includes reducing and improving the fat content (replacing beef fat by konjac gel) and incorporating olive oil (replacing beef fat by olive oil stabilized in a konjac matrix) and also reducing sodium by replacing sodium chloride with a mixture of salts containing potassium chloride, calcium chloride and magnesium chloride. A preservative (sodium metabisulphite) was also used to improve the shelf-life of the product. The modified fat content formulation was selected as the most appropriate in compositional, technological and sensory terms from those described by Triki et al. (2013).

2. Materials and methods

2.1. Materials and konjac material preparation

Fresh post-rigour beef meat (20.6%, 6.1%, 71.7% protein, fat and moisture contents respectively) and beef fat (10.0%, 46.1% and 37.3% protein, fat, and moisture contents respectively) were obtained from a local market, minced (15 mm diam. hole mincer plate) (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy), and frozen at −20 °C. Frozen storage did not exceed 14 days.

The two types of konjac materials (konjac gel: KCC and olive oil-in-konjac matrix:OKCCM) used as fat analogues, were prepared in duplicate as reported by Triki et al. (2013). Briefly, KCC and OKCCM were prepared with konjac flour (glucomannan 83%, 120 mesh, from Trades S.A. Barcelona, Spain) (5.0%) homogenised (Stephan Universal Machine UM5, Stephan Machinery GmbH and Co., Hameln, Germany) with 64.8% of the water and i-carrageenan (Hispanagar S.A, Burgos, Spain) (1.0%) and 20% w/w of olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain) in the case of OKCCM. Then the mixtures were homogenised with pre-gelled cornstarch powder (Amigel, Julio Criado, S.L. Madrid. Spain) (3.0%) previously dispersed in 16.2% of water. The mixture was cooled to 10 °C and 10% of a $Ca(OH)_2$ solution of 1% was added with gentle stirring, then they were placed in suitable containers, covered, manually overpressured to eliminate air and stored at 2 ± 2 °C until used (within 24 h of preparation).

2.2. Experimental design and manufacture of merguez sausage

Merguez sausages were designed and formulated to modified fat content (Triki et al., 2013) and reduced sodium level, using similar amounts of lean meat. Fat reduction was carried out by replacing the "beef fat" with the same proportion of two fat analogues (KCC and OKCCM). Six different formulations of merguez were made up (Table 1). Two normal fat content formulations were prepared: one control sample with normal sodium content (CNS with 1.4% NaCl), and another control sample with reduced sodium content (CRS with 1.4% mixture of salt – MS – containing 0.7% NaCl, 0.35% KCl, 0.20% $CaCl_2$ and 0.15% $MgCl_2$). The modified fat content formulations were selected as the most appropriate in compositional, technological and sensory terms from those described by Triki et al. (2013). Two low fat formulations were prepared, replacing all "beef fat" by the same proportion of konjac gel (KCC fat analogue): one low fat sample with normal sodium content (LFNS containing 1.4% NaCl) and one low fat sample with reduced sodium content (LFRS) with the same proportion of MS mixture as above. Two formulations with reduced fat content and modified fatty acid profile were prepared by partial replacement of "beef fat" with olive oil-in-konjac matrix (OKCCM fat analogue): one reduced fat sample with normal sodium content (RFNS containing 1.4% NaCl) and the other reduced fat sample with reduced sodium content (RFRS) with the same proportion of the MS mixture as above. According to the experimental design, for each fat content, formulations containing normal reduced sodium levels were prepared. Compared with the formulations containing normal salt content (CNS, RFNS and LFNS), the reduced salt content formulations (CRS, RFRS and LFRS) were designed to contain 50% of the normal sodium level. On the other hand, in the CNS, CRS, LFNS and LFRS formulations all the fat is from beef, while in the RFNS and RFRS formulations, part of the fat content is olive oil (Table 1). The sausage production conditions were described by Triki et al. (2013). Each formulation was duplicated. Briefly, meat and fat, previously thawed (18 h at 2 ± 2 °C) together with the konjac materials, were minced at particle size of 15 mm (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy) and placed in a mixer (MAINCA, Granollers, Barcelona, Spain). Half of the water and additives (Table 1) were added to the mixture and mixed for 1 min. After this, the other half of the additives was added and the whole mixed again for 2 min. The final temperature of the meat batter was between 3–6 °C. The sausage mixture was immediately stuffed into 22 mm-diametre natural lamb casings (Type C-20/22 Julio Criado Gómez S.A., Spain) using a stuffer (MAINCA, Granollers, Barcelona, Spain). Sausages were handlinked to 10 ± 2 cm and the resulting strings of sausages were covered with plastic and placed in a room at 2 °C overnight for ingredient equilibration (stabilization). After that, the sausages were placed on EPS trays (Type 89 white SPT—Linpac Packaging Pravia, S.A. N R.G.S., Spain), covered with oxygen-permeable cling film (LINPAC Plastics, Pontivy, France) in aerobic conditions and kept at 2 °C. Samples from each batch were periodically taken for analysis (days 0, 3, 6, 10) in order to monitor the storage effect on quality characteristics.

Table 1
Formulation (%) of fresh merguez sausages.

	Beef meat	Beef fat	KCC	OKCCM	NaCl	MS		
						KCl	$CaCl_2$	$MgCl_2$
CNS	55.00	29.00	–	–	1.4	–	–	–
CRS	55.00	29.00	–	–	0.7	0.35	0.20	0.15
RFNS	55.00	7.25	–	21.75	1.4	–	–	–
RFRS	55.00	7.25	–	21.75	0.7	0.35	0.20	0.15
LFNS	55.00	–	29.00	–	1.4	–	–	–
LFRS	55.00	–	29.00	–	0.7	0.35	0.20	0.15

Sample denomination: CNS and CRS. – control sample (C) (all beef fat) prepared with normal fat content with normal sodium chloride content (NS) and sodium chloride reduced to half (RS) and replaced by a mixture of salts (MS) respectively; RFNS and RFRS sausages prepared replacing 75% of beef fat by the same proportion of OKCCM (oil-in-konjac matrix, as konjac material containing 20% of olive oil) with NS and RS and replaced by MS respectively; LFNS and LFRS-sausage prepared replacing 100% of beef fat by the same proportion of KCC (konjac gel) with NS and RS and replaced by MS respectively.
11% of water, 0.5 coriander (Naturel, Conditionnement de produits agricoles, El Sahlin, Tunisia), 0.8% fennel (Kamy S.A. Nabeul, Tunisia), 0.2% hot pepper (Jose M[a] Fuster Hernandez S.A., Murcia, Spain), 0.2% paprika (Jose M[a] Fuster Hernandez S.A., Murcia, Spain), 0.2% mint (DUCROS, Mac Cormick S.A., Spain), 2.0% harissa (Ferrero, TUCAL S.A., Manouba, Tunisia) and 0.045% preservative $Na_2S_2O_5$ (sodium metabisulphite) (Manuel Riesgo, S.A., Madrid, Spain) were also added to all the samples. Sodium and potassium chloride (Panreac Química, S.A. Barcelona, Spain), calcium and magnesium chloride (Manuel Riesgo, S.A., Madrid, Spain).

2.3. Proximate analysis and mineral contents

Sample moisture and ash contents (%) were determined (AOAC, 2005) in triplicate in all fresh merguez sausage. Protein content (%) was measured in quadruplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). Fat content (%) was evaluated in triplicate according to Bligh and Dyer (1959). Carbohydrates were estimated taking into account ingredient composition and formulation. The energy content was estimated based on the accepted levels of 9.1 kcal/g for fat and 4.1 kcal/g for protein and carbohydrates.

Ca, Mg, Na, K and Fe contents (mg/100 g) were determined in fresh merguez sausages as reported by Serrano et al. (2005) on an atomic absorption spectrophotometer (Perkin–Elmer, Model 5100, Norwalk, Connecticut. USA) and determined in triplicate.

2.4. Sensory evaluation

Merguez sausages were assessed by a 20 member panel as described by Triki et al. (2013). For sensory analysis, which was carried out the day after preparation (day 0), the merguez sausages were heated in porcelain dishes for 2 min (60 s on each side) in a microwave (Saivod, Spain) at 70 °C (1200 W). Then the sausages were cut with a knife into 3 cm long portions, placed on plates and served to the panellists. The panellists evaluated the sausages on the following parameters on a scale of 0–10: juiciness, firmness and general acceptability. The sensory analysis was carried out three times for the different samples and at the very outset of the storage.

2.5. Purge and cooking losses

The purge loss (PL) was evaluated in quadruplicate during storage of the merguez sausages. Three trays of sausages from each formulation were tempered for 20 min (at room temperature). After the sausages were removed from the package, their surfaces were wiped with a paper towel to eliminate the superficial exudate (tiny drops) before weighting them. The purge loss was calculated by the weight difference and expressed as a percentage of the initial weight.

The cooking loss was studied as total, water and fat loss of the merguez sausages, in order to understand the water and fat binding properties associated with the cooking processing. To analyse these properties, around 32 g from each formulation was placed in containers (27 mm diameter) and hermetically closed and heated (70 °C/30 min) in a water bath (GRANT, OLS 200, Grant instruments, Cambridge, Ltd., England). When heating was complete, the containers were opened and left to stand upside down (for 30 min) to release the exudate onto a previously weighed plate. Cooking loss (CL) was expressed as a % of the initial sample weight. Water loss (WL) was determined as weight loss after heating the total fluid released (in the plate used for cooking loss), for 16 h at 105 °C in a drying oven (Model IDL-AI-36, Labolan SL, Navarra, Spain) and was expressed as a % of the initial sample weight. Fat loss (FL) was calculated as the difference between CL and WL. Three determinations for each sample were carried out.

2.6. Lipid oxidation

Oxidative stability was evaluated from changes in thiobarbituric acid-reactive substances (TBARS) in the fresh merguez sausages during storage. The procedure for measurement of TBARS was based on methods used by López-López, Cofrades, Yakan, Solas, and Jiménez-Colmenero (2010). The results were expressed as mg malonaldehyde/kg of sample. TBARS determinations were performed three times.

2.7. Compression/extrusion tests

Compression/extrusion tests were carried out using a miniature Kramer shear/Ottawa cell. A compression plate was used to perform the compression/extrusion analysis. The measurements were performed on the fresh merguez sausages discarding the external sausage casing. Samples were cut approx. 2.5 cm long. A 5 kg load cell was used. The force was exerted at 50% deformation at 2 mm/s crosshead speed using a TA.XT2i Stable Micro Systems Texture Analyser (Stable Microsystems Ltd., Surrey, England), with the Texture Expert programme. Maximum force (extrusion force, N) provides an indication of sample consistency or firmness. Determinations were carried out six times at room temperature (22 °C).

2.8. Colour measurement and pH

Colour (CIE-LAB tristimulus values, lightness, L*; redness, a* and yellowness, b*) was evaluated on a Chroma Meter CR-400 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). Determinations were carried out on cross-sections of the fresh merguez sausages. Fifteen determinations were performed from each formulation.

The pH was determined using a pH meter (827 pH Lab Methrom, Herisau, Switzerland) on 10 g homogenate samples in 100 ml of distilled water. Three measurements were performed per sample.

2.9. Microbiological analysis

10 g of each fresh merguez sausages (from 2 trays per sample) was taken and placed in sterile plastic bags with 90 ml of peptone water (0.1%) with 0.85% NaCl. After 2 min in a stomacher blender (Stomacher Colworth 400, Seward, UK), appropriate decimal dilutions were pour-plated (1 ml) on the following media: Plate Count Agar (PCA) (Merck, Germany) for the total viable count (TVC) (30 °C for 72 h); De Man, Rogosa, Sharpe Agar (MRS) (Merck, Germany) for lactic acid bacteria (LAB) (30 °C for 3–5 days); and Violet Red Bile Glucose Agar (VRBG) (Merck, Germany) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of colony-forming units per gram (log cfu/g).

2.10. Analysis of biogenic amines by ion-exchange chromatography

The extraction and analysis with HPLC of tyramine, histamine, phenylethylamine, putrescine, cadaverine, tryptamine, agmatine, spermidine and spermine in the fresh merguez sausages were performed as described by Triki, Jiménez-Colmenero, Herrero, and Ruiz-Capillas (2012). The results are averages of at least 3 determinations.

2.11. Statistical analysis

One-way analyses of variance (ANOVA) to evaluate the statistical significance ($P < 0.05$) of the effect of merguez sausage formulation and two-way ANOVA as a function of formulation and storage time were performed. Least squares differences were used for comparison of mean values among formulations and Tukey's HSD test to identify significant differences ($P < 0.05$) between formulations and storage times. In addition, Pearson product moment correlation (r) was performed to determine the relationships between parameters. The software used was SPSS 14.0 (SPSS Inc, Chicago, USA).

3. Results and discussion

3.1. Proximate analysis, energy value and mineral content

The fat content of the merguez sausages ranged from 3.30 to 16.71% (Table 2) and as expected, three different fat levels were observed. Compared with the normal fat sample, total replacement of beef fat by konjac gel (LFNS and LFRS) yielded products with the lowest ($P < 0.05$) fat content (3.30% and 3.90%, respectively), representing fat reduction of 75.7–79.4%. However, when beef fat in the RFNS and RFRS was partially replaced by an olive oil-in-konjac matrix (OKCCM), the fat reduction was less (32.9% and 37.7% respectively), due to the presence of olive oil. In the RFNS and RFRS samples, 24.5% and 25.8% respectively of total fat come from olive oil (Table 2). These results agree with those reported by Triki et al. (2013).

The protein content ranged from 12.3 to 15.8%, with the highest ($P < 0.05$) values for the control product, and no differences ($P > 0.05$) between reformulated samples (Table 2). This could be due to the preparation of all the samples with the same meat content. On the other hand there is a lower protein contribution from the beef fat as a result of the reformulation strategy (Table 1). The moisture percent of the products

Table 2
Proximate analysis (%), energy values (kcal/100 g) and mineral content (mg/100 g) of fresh merguez sausages.

	CNS	CRS	RFNS	RFRS	LFNS	LFRS
Moisture	61.99 ± 0.67^{b}	60.61 ± 0.42^{a}	68.94 ± 0.24^{c}	70.23 ± 0.25^{d}	78.35 ± 0.03^{e}	77.28 ± 0.71^{e}
Fat	16.04 ± 0.74^{c}	16.71 ± 0.62^{c}	10.77 ± 0.13^{b}	9.99 ± 0.28^{b}	3.30 ± 0.14^{a}	3.90 ± 0.07^{a}
Protein	15.79 ± 0.82^{b}	15.83 ± 0.49^{b}	13.48 ± 0.17^{a}	13.36 ± 0.50^{a}	12.95 ± 0.89^{a}	12.30 ± 0.49^{a}
Ashes	2.44 ± 0.02^{bc}	2.38 ± 0.00^{ab}	2.53 ± 0.01^{c}	2.30 ± 0.11^{a}	2.51 ± 0.03^{c}	2.51 ± 0.03^{c}
Fat reduction (%)	–	–	32.9	37.7	79.4	75.7
Energy value	210.7	217.0	161.3	153.7	91.1	93.9
From total fat	146.0 (69.3)	152.1 (70.1)	98.0 (60.8)	90.9 (59.1)	30.0 (32.9)	35.5 (37.8)
From beef fat	146.0 (69.3)	152.1 (70.1)	58.4 (36.2)	51.3 (33.4)	30.0 (32.9)	35.5 (37.8)
From olive oil*	–	–	39.6 (24.5)	39.6 (25.8)	–	–
Sodium	630.7 ± 12.01^{b}	391.0 ± 20.98^{a}	643.8 ± 11.84^{b}	386.3 ± 15.72^{a}	649.8 ± 21.96^{b}	412.0 ± 13.50^{a}
Potassium	321.1 ± 19.35^{a}	532.1 ± 15.08^{b}	312.9 ± 11.57^{a}	518.5 ± 5.71^{b}	308.8 ± 2.80^{a}	523.6 ± 18.85^{b}
Calcium	26.53 ± 0.73^{a}	81.75 ± 1.07^{c}	34.88 ± 1.48^{b}	100.01 ± 4.50^{d}	39.07 ± 2.03^{b}	96.88 ± 0.24^{d}
Magnesium	23.27 ± 0.04^{b}	42.99 ± 0.38^{e}	23.19 ± 1.11^{ab}	40.98 ± 1.01^{d}	21.22 ± 0.74^{a}	38.29 ± 0.94^{c}
Iron	2.39 ± 0.05^{a}	2.15 ± 0.06^{a}	2.24 ± 0.17^{a}	2.33 ± 0.14^{a}	2.25 ± 0.17^{a}	2.38 ± 0.16^{a}

For sample denomination see Table 1.
Means ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).
* Calculated on the basis of formulation (20% of OKCCM added is olive oil).

(Table 2) showed some differences ($P < 0.05$) according to the formulation. The highest ($P < 0.05$) levels (77.28% and 78.35%) were observed in the LFRS and LFNS formulations respectively, followed by reduced fat and control samples. An inverse relationship was therefore observed between fat and moisture content (Table 2). This is related to the reformulation strategy (dilution effect), replacing beef fat by konjac gel (containing 90% water). However no clear relationship between sodium chloride reduction and moisture content was observed (Table 2). Ash contents ranged from 2.30 to 2.53% with no effect of the formulation in quantitative terms (Table 2).

The energy content of the control samples CNS and CRS was 210.7 and 217.0 kcal/100 g respectively (around 70% from beef fat) while in the reformulated samples it ranged from 91.1 to 161.3 kcal/100 g, with fat accounting for between 33% and 61% in LFNS and RFNS, respectively (Table 2). In the RFNS and RFRS samples with partial beef fat replacement by OKCCM, olive oil accounted for 24.5% and 25.8% respectively of the total energy content. Percent reductions in the energy content of merguez compared with control samples were higher in LFNS and LFRS (over 55%). Similar energy reduction levels were reported by Osburn and Keeton (1994) and Triki et al. (2013).

As expected, mineral values of the different samples were affected ($P < 0.05$) by the formulation (Table 2). Sodium content in reduced-salt batches (CRS, RFRS and LFRS), ranging between 386.3 and 412 mg/100 g, was almost half that of the normal-salt samples (CNS, RFNS, LFNS). A reduction of more than 36% sodium was achieved in the reduced sodium formulated samples (RS), an important advance in terms of health and nutritional considerations (Desmond, 2006; NAOS, 2012; WHO/FAO, 2011).

On the other hand, an inverse relationship between potassium and sodium contents was observed. The K levels ranged from 518.5 to 532.1 mg/100 g for RFRS, LFRS, CRS versus 308.8 and 321.1 mg/100 g for LFNS, RFNS, CNS samples ($P < 0.05$). Potassium is fundamental in a significant number of body processes, including fluid balance, protein synthesis, nerve conduction, energy production, muscle contraction, synthesis of nucleic acids and regulating heart rate (WHO/FAO, 2011). The reformulated merguez provided 10–15% of the daily potassium intake, as the recommended daily amount (RDA) of potassium for a normal adult is 4700 mg.

It is also important to take the sodium–potassium ratio into account, as it influences the regulation of high blood pressure. Various studies have shown that it is not just the quantities of these two nutrients which are important, but their ratio. A 2:1 intake of potassium to sodium may lower the mortality risk from cardiovascular disease by 50%; ratios of above 1:4 present high cardiovascular risk (Stobbe, 2011; Yang et al., 2011). The reformulated merguez sausages present a K/Na ratio nearer to the recommended 2:1 with a ratio of 1.30 in the reduced salt samples and around 0.50 in the batches with normal salt (NaCl) levels. The salt levels in these batches exceed those considered as high risk.

Calcium and magnesium levels display the same behaviour as the potassium levels in the formulations. This was expected, due to the substitution of 14.3% and 10.7% of sodium chloride by calcium and magnesium chloride, respectively. A significant difference was observed in calcium levels, between normal-salt samples (26.53–39.07 mg/100 g) and reduced-salt samples (81.75–100.01 mg/100 g), and in magnesium levels, between normal-salt samples (21.22–23.27 mg/100 g) and reduced-salt samples (38.29–42.99 mg/100 g). Meat and meat products are generally poor in calcium, 4–21 mg/100 g in beef (USDA, 2004). The RDA for daily calcium intake is 1000–1300 mg. An adequate calcium intake is essential for the development of strong, healthy bones during adolescence. 1200–1250 mg Ca is recommended for the elderly, people on low fat diets, pregnant women, people suffering from stress (which increases excretion) and menopausal women (Musaiger, Hassan, & Obeid, 2011). In the present study, 100 g of the reformulated merguez (CRS, RFRS, LFRS) can provide 8–10% of the total calcium daily intake for people requiring additional calcium. Another advantage of the addition of dietary calcium is that it binds to heme iron, suppressing its toxicity (Toldrá & Reig, 2011).

In the reformulated merguez, magnesium is closely balanced with calcium. The RDA for Mg is 240–360 mg/day. According to ANSES (2008), merguez sausage contains 21.1 mg/100 g. In the present study, this was increased to approx. double in the reformulated merguez sausage, providing 10–20% of RDA of this mineral. Health benefits of magnesium include alleviating or preventing osteoporosis, heart attacks, hypertension, constipation, migraine, leg cramps, kidney stones and gallstones (Carpenter et al., 2006).

No significant differences were observed in iron levels between the formulations, with levels ranging from 2.15 to 2.39 mg/100 g, similar to the amounts reported by Triki et al. (2013). The iron provided by these products represents 15% of the RDA (14 mg/day). Because meat is the main source of iron, bioavailability is assured, and hence the new product may have a major impact on groups vulnerable to iron deficiency, one of the most prevalent nutritional deficiencies in both developing and developed countries (Neumann, Harris, & Rogers, 2002).

3.2. Sensory evaluation

Table 3 shows the sensory evaluation of the sausages. No significant differences were observed in sensory parameters (juiciness, firmness and general acceptability) as affected by formulation (modified fat content and reduced salt). Various authors (Ruusunen et al.,

Table 3
Sensory evaluation of cooked merguez sausages on day 0 of refrigerated storage.

Samples	Juiciness	Firmness	General acceptability
CNS	6.02 ± 1.38^{a}	5.43 ± 1.11^{b}	6.17 ± 1.21^{a}
CRS	6.59 ± 0.92^{b}	5.21 ± 1.41^{ab}	6.14 ± 1.03^{a}
RFNS	6.26 ± 1.31^{a}	3.48 ± 0.68^{a}	5.70 ± 1.15^{a}
RFRS	5.84 ± 1.13^{a}	4.94 ± 1.55^{ab}	5.55 ± 1.28^{a}
LFNS	5.78 ± 1.28^{a}	4.23 ± 1.02^{ab}	5.39 ± 1.03^{a}
LFRS	4.82 ± 1.18^{a}	5.00 ± 1.04^{ab}	4.75 ± 1.23^{a}

For sample denomination see Table 1. Means ± standard deviation. Different letters in the same column indicate significant differences ($P < 0.05$).

2005; Tobin et al., 2012) have suggested that fat and salt act as flavour enhancers, increasing the intensity of the flavour of meat products and therefore salt and fat reductions reduce the perceived saltiness and palatability and also weaken the overall flavour in meat products. In this study, the changes in product composition associated with the type of analogue used to condition the content and type of lipid material, and the sodium reduction strategy used, did not result in any limitations in the sensory appreciation of the products. This may be explained partly by the replacing of NaCl by other salts and the use of large amounts of spices in the formulation (Triki et al., 2013). Other authors (Desmond, 2006; Zanardi et al., 2010) have observed that replacing sodium chloride by other salts affects sensory parameters, with bitter or metallic flavours detected with the substitution of 40% by KCl. In this study, a mixture of three compounds (KCl, $MgCl_2$ and $CaCl_2$) was used, which allowed low concentrations of each to be used (25% KCl, 14.3% $CaCl_2$ and 10.7% $MgCl_2$). The effect on the sensory parameters is therefore less than reported in fermented products (Desmond, 2006; Zanardi et al., 2010), but similar to those reported by Pasin et al. (1989), who observed that NaCl can be reduced by 75% in fresh pork sausage patties and replaced by KCl without affecting the hedonic rating for the products.

The use of SO_2 in all the formulations does not appear to have any negative effect in sensory terms. Mathenjwa, Hugo, Bothma, and Hugo (2012), observed that fresh sausages treated with preservatives were preferred by consumers.

The results of this study indicate that the strategies used to modify fat content and reduce sodium had no negative effect on the sensory quality of the healthier merguez.

3.3. Purge and cooking losses

Purge loss (PL) of the merguez was not affected ($P > 0.05$) by formulation, but was affected ($P < 0.05$) by refrigerated storage (data not shown). Initial levels ranged from 1.61 to 2.53%, which increased ($P < 0.05$) to 2.71–3.72% by the end of storage. These results agree with those of Triki et al. (2013) and show that strategies for improving fat content and reducing sodium did not negatively affect the purge loss. PL is an important parameter as it influences product appearance, consumer perception, and stability.

Cooking loss measures the ability of the system to bind water and fat after protein denaturation and aggregation. Cooking loss (total, water and fat loss) was affected ($P < 0.05$) by the formulation and storage, with interaction ($P < 0.05$) between both factors (Table 4). In general, the formulations with reduced sodium content (CRS, RFRS and LFRS) had higher ($P < 0.05$) cooking loss as compared with the same samples with normal sodium content (CNS, RFNS and LFNS); this was more evident during storage (Table 4). Others have observed greater cooking loss in low-salt restructured poultry (Cofrades, López-López, Ruiz-Capillas, Triki, & Jiménez-Colmenero, 2011; Ruusunen et al., 2005). In this study it was also observed that the samples with higher fat contents presented greater cooking loss (Table 4). These results are similar to those observed by Ruusunen et al. (2005) in ground meat patties reformulated with different fat and sodium levels.

Table 4
Cooking loss (total loss, water loss and fat loss) of the merguez sausages during refrigerated storage.

	Storage time (days at 2 °C)			
	0	3	6	10
Total loss (%)				
CNS	21.67 ± 1.80^{c2}	18.48 ± 0.89^{b2}	16.66 ± 0.70^{b12}	12.07 ± 1.52^{a1}
CRS	25.06 ± 2.20^{c3}	23.49 ± 0.45^{bc3}	21.98 ± 0.61^{b4}	14.56 ± 1.51^{a12}
RFNS	18.21 ± 0.67^{c1}	15.56 ± 1.70^{bc1}	15.19 ± 1.09^{b1}	11.92 ± 2.06^{a1}
RFRS	18.99 ± 0.30^{b1}	18.72 ± 0.50^{ab2}	19.21 ± 0.12^{b23}	16.14 ± 0.86^{a2}
LFNS	18.54 ± 0.92^{ab1}	18.98 ± 0.60^{ab2}	19.38 ± 1.24^{b234}	16.80 ± 1.13^{a2}
LFRS	19.98 ± 0.52^{a12}	22.04 ± 0.40^{b3}	21.21 ± 1.91^{ab34}	22.40 ± 1.45^{b3}
Water loss (%)				
CNS	14.97 ± 1.11^{c1}	13.39 ± 0.59^{bc1}	12.22 ± 0.35^{b1}	9.70 ± 1.06^{a1}
CRS	16.89 ± 1.45^{b123}	16.53 ± 0.29^{b2}	15.98 ± 0.51^{b23}	10.77 ± 0.99^{a1}
RFNS	16.18 ± 0.71^{c12}	13.64 ± 1.67^{b1}	13.56 ± 1.00^{b12}	10.53 ± 1.88^{a1}
RFRS	17.27 ± 0.26^{b23}	16.88 ± 0.44^{b2}	17.30 ± 0.13^{b3}	14.50 ± 0.82^{a2}
LFNS	17.27 ± 0.85^{b23}	17.59 ± 0.56^{b2}	17.90 ± 1.13^{b34}	15.49 ± 1.05^{a2}
LFRS	18.65 ± 0.26^{a3}	20.35 ± 0.38^{b3}	19.60 ± 1.79^{ab4}	20.69 ± 1.36^{b3}
Fat loss (%)				
CNS	6.71 ± 0.69^{c3}	5.09 ± 0.33^{b2}	4.44 ± 0.28^{b2}	2.37 ± 0.50^{a2}
CRS	8.17 ± 0.76^{d4}	6.96 ± 0.37^{c3}	6.00 ± 0.10^{b3}	3.79 ± 0.54^{a3}
RFNS	2.03 ± 0.03^{b2}	1.93 ± 0.10^{b1}	1.63 ± 0.19^{a1}	1.40 ± 0.21^{a1}
RFRS	1.73 ± 0.09^{ab12}	1.84 ± 0.06^{bc1}	1.91 ± 0.01^{c1}	1.64 ± 0.04^{a1}
LFNS	1.27 ± 0.08^{a1}	1.39 ± 0.04^{a1}	1.47 ± 0.11^{a1}	1.30 ± 0.07^{a1}
LFRS	1.34 ± 0.07^{a1}	1.69 ± 0.01^{b1}	1.61 ± 0.14^{b1}	1.71 ± 0.10^{b1}

For sample denomination see Table 1. Means ± standard deviation. Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).

Cooking loss decreased ($P < 0.05$) during refrigerated storage, although was not observed in low-fat samples (LFNS and LFRS). In agreement with these results, it has been reported that cooking loss increased with the proportion of konjac gel in low-fat fresh sausages reformulated with konjac (Osburn & Keeton, 1994). However, small differences in cooking loss (27–29%) as affected by fat content (5–29%) were found in breakfast sausages (Barbut & Mittal, 1995). In contrast to the difference observed in this experiment, Triki et al. (2013) reported that cooking loss (total loss) of merguez sausages increased during refrigerated storage. These apparently conflicting results may be due to the influence of two main factors: in the present work pH levels (see below) were not affected by storage, in contrast to the earlier study (Triki et al., 2013) where the pH decreased over storage. It is well known that water binding properties of meat systems decrease with pH. Reducing the NaCl content increased cooking losses, with higher losses as the fat content decreased. This has been reported many times (Hayes, Stepanyan, Allen, O'Grady, & Kerry, 2011; Lyons, Kerry, Morrissey, & Buckley, 1998; Osburn & Keeton, 1994; Toldrá, 2002).

As expected, percentage water loss and total loss, showed the same behaviour in the different formulations and during storage. Higher water losses ($P < 0.05$) were observed at the start of the experiment in the reformulated samples (16.18–18.65%) compared with the control samples (14.97–16.89%), due to the lower moisture and fat content of these samples compared with the reformulated ones (Table 2). The use of konjac in the reformulated sausages should also be taken into account, as it is related to higher cooking loss (Osburn & Keeton, 1994; Triki et al., 2013). At the end of storage, the highest water losses were recorded in LFRS samples (20.69%), increasing ($P < 0.05$) throughout the experiment, in contrast to the other samples which showed a significant decrease. As expected, correlation ($P < 0.01$) ($r = +0.81$) between water loss and moisture levels was observed and between fat content and fat loss ($P < 0.01$) ($r = +0.730$ and $r = +0.878$) during storage. The highest fat losses ($P < 0.05$) were observed in CNS and CRS samples (6.71 and 8.17% respectively), formulated with the highest fat contents (Table 2). At the end of storage the fat loss in these samples was 2.37% and 3.79%

respectively, higher levels of fat loss than observed in the reformulated samples at the start of storage, 1.27–2.03%. Similar results were described by Triki et al. (2013) who observed that fat loss was generally influenced more by the fat level of the sausages than by the type of replacement (KCC and OKCCM).

3.4. Lipid oxidation (TBARs)

TBARS values of merguez sausages were not affected ($P > 0.05$) by formulation and storage (data not shown), ranging between 0.06 and 0.15 mg MDA/kg of sample. No effects of salt type were observed in the sausages, in spite of the different pro-oxidative effects of the different salts used, greater in the case of NaCl (Horita, Morgano, Celeghini, & Pollonio, 2011; Zanardi et al., 2010). Replacing NaCl by other salts decreased lipid oxidation in ground pork (Hernández, Park, & Soon Rhee, 2002; Zanardi et al., 2010). The low rate of lipid oxidation may be due to the presence of antioxidant in some of the spices added to all formulations, the presence of sodium metabisulphite and the refrigerated storage conditions (Ruiz-Capillas & Jiménez-Colmenero, 2009; Triki et al., 2013). Mathenjwa et al. (2012) showed that sodium metabisulphite reduced TBARS levels by half (≈0.2 mg MDA/kg) after 9 days of refrigerated storage in *boerewors* fresh sausages.

3.5. Compression/extrusion tests

Extrusion force (EF) values of merguez sausages are shown in Table 5. This parameter showed significant differences ($P < 0.05$) between formulations and storage time with interaction ($P < 0.05$) between both factors. Sausages in which all the beef fat was replaced with konjac gel (LFNS and LFRS) presented the lowest ($P < 0.05$) EF. These results indicated lower consistency in samples formulated with konjac gel. This is in line with the results obtained by Osburn and Keeton (1994) who reported that in low-fat fresh pork sausages shear force decreased when konjac flour gel levels increased. Compared to normal fat sausages (CNS and CRS), EF values were not affected ($P > 0.05$) by partial beef fat replacement with olive oil-in-konjac matrix (RFNS and RFRS). The different textural behaviours observed between samples with total or partial replacement, could be related to the nature of the konjac material and the proportion of fat replaced (Jiménez-Colmenero et al., 2010; Kao & Lin, 2006; Lin & Huang, 2003; Osburn & Keeton, 2004). A correlation was established between EF ($P < 0.05$; $r = +0.82$) and fat content of merguez sausages, where higher fat content implies harder sausage (high EF values). No differences ($P > 0.05$) were observed in EF as a result of reducing the sodium level (Table 5).

Changes in the EF of merguez sausages during storage were influenced by formulation (Table 5). In normal fat content formulations (CNS and CRS) EF increased ($P < 0.05$) during refrigerated storage. This was less ($P < 0.05$) pronounced in samples with reduced sodium level (CRS). There was no clear trend during refrigerated storage in sausages with total or partial fat replacement. In samples with partial replacement of fat by olive oil-in-konjac matrix (RFNS and RFRS) the EF values were lower on day 10 of storage, regardless of the sodium content. A different behaviour was observed in sausages with total fat replacement by konjac gel. These samples (LFRS, LFNS) showed a significant increase in EF on day 10 of storage only when the sodium level was reduced.

Table 5
Extrusion force (N/g) of the fresh merguez sausage during refrigerated storage.

	Storage time (days at 2 °C)			
Extrusion force (N/g)	0	3	6	10
CNS	1.10 ± 0.35^{a2}	2.02 ± 0.41^{b3}	1.68 ± 0.49^{a2}	2.70 ± 0.32^{c3}
CRS	1.29 ± 0.39^{a2}	1.06 ± 0.43^{a1}	1.98 ± 0.72^{b2}	2.05 ± 0.60^{b2}
RFNS	1.38 ± 0.13^{b2}	1.56 ± 0.26^{b2}	0.95 ± 0.17^{a1}	0.63 ± 0.17^{a1}
RFRS	1.17 ± 0.43^{b2}	0.97 ± 0.36^{b1}	1.66 ± 0.24^{c2}	0.51 ± 0.13^{a1}
LFNS	0.57 ± 0.26^{a1}	1.01 ± 0.27^{b1}	0.69 ± 0.13^{a1}	0.49 ± 0.10^{a1}
LFRS	0.54 ± 0.06^{a1}	0.75 ± 0.15^{a1}	0.95 ± 0.16^{c1}	0.76 ± 0.14^{b1}

For sample denomination see Table 1. Means ± standard deviation. Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).

3.6. Colour

The results for the colour parameters of fresh merguez sausage during storage are presented in Table 6. They were affected ($P < 0.05$) by formulation and storage with interaction ($P < 0.05$) between both factors.

In general, initially it was observed that the low fat batches, regardless of the salt level (LFNS, LFRS) had lower L* and b* values ($P < 0.05$), while a* (degree of redness) was not significantly different between batches. CNS and CRS presented the highest initial levels ($P < 0.05$) of lightness (49.18 and 51.35, respectively) followed by RFRS, RFNS, LFRS and LFNS, due to the presence of whiter (animal) fat. Similar initial colour values were observed in merguez sausages (Triki et al., 2013) and other raw fresh meat products (Barbut & Mittal, 1995; Hayes et al., 2011; Osburn & Keeton, 1994). The addition of konjac tended to decrease L* values (Barbut & Mittal, 1995; Osburn & Keeton, 1994; Triki et al., 2013). Toldrá (2002), linked the presence of NaCl in meat products to their luminosity. In the present study, the colour parameters are generally more influenced by the fat type and content than by the different salts used in the formulation.

During storage L* and b* values were relatively constant ($P > 0.05$), but a* decreased ($P < 0.05$) at the end of storage (Table 6), possibly due to loss of colour of ingredients such as harissa, paprika and red hot pepper. This agrees with other experiment reported for fresh sausages stored at 4 °C (Boles, Mikkelsen, & Swan, 1998; Hayes et al., 2011; Mathenjwa et al., 2012; Triki et al., 2013). Triki et al. (2013) observed a pronounced decrease in redness during chilled storage of merguez. However, in the present study, the loss of a* is less pronounced (only seen after 10 days) and this may be due to the use of sulphites in the formulation. These preservatives help to stabilize the product colour and inhibit discoloration, due to their antioxidant activity (Ruiz-Capillas & Jiménez-Colmenero, 2009).

3.7. pH

The pH of the fresh merguez sausage were not affected ($P > 0.05$) by formulation and storage time (data not shown), with values ranging from 5.62 to 5.82. The initial levels were similar to those observed in fresh sausages (Triki et al., 2013). In this type of product a decrease in pH is normally observed, due mainly to microorganism growth, especially lactic acid bacteria, with the most pronounced decrease in batches containing konjac (Benkerroum et al., 2003; Triki et al., 2013). In the present study, the pH did not change significantly throughout storage, due mainly to the preservative effect of sodium metabisulphite added to all formulations, which led to low microbial growth, including lactic acid bacteria (see below).

3.8. Microbiology

Microbiological counts of the fresh merguez sausage are shown in Table 7. Initially, higher levels of total viable counts, lactic acid bacteria and *Enterobacteriaceae* were observed in the control samples (CNS) with 5.88, 5.60 and 4.15 log cfu/g, respectively. The TVC levels in this formulation were below the legal limit of 6 log cfu/g which is the acceptable total microbial quality standard for fresh sausages. In general, the other samples had no initial microbial variations ($P > 0.05$), with no clear influence of fat reduction, modification or replacement of NaCl by other salts. These results are similar to those reported by others for this type of product (Mathenjwa et al., 2012;

Table 6
Colour parameters (lightness, L*; redness, a*; yellowness, b*) of the merguez sausages during refrigerated storage.

Parameters	Sample	Storage time (days at 2 °C)			
		0	3	6	10
L*	CNS	49.18 ± 1.38^{a23}	50.40 ± 2.61^{a2}	49.86 ± 3.43^{a34}	50.41 ± 2.00^{a4}
	CRS	51.35 ± 3.48^{ab3}	49.57 ± 1.50^{a2}	50.39 ± 2.24^{ab4}	52.12 ± 3.31^{b4}
	RFNS	47.58 ± 1.03^{a2}	47.73 ± 2.17^{a2}	47.13 ± 1.65^{a2}	46.41 ± 1.36^{a23}
	RFRS	48.54 ± 2.41^{a2}	48.69 ± 2.25^{a2}	47.37 ± 0.87^{a23}	47.85 ± 1.39^{a3}
	LFNS	41.49 ± 1.17^{a1}	41.71 ± 1.27^{a1}	42.82 ± 1.46^{a1}	43.47 ± 1.25^{a1}
	LFRS	43.31 ± 1.62^{a1}	43.68 ± 1.13^{a1}	43.65 ± 2.30^{a1}	45.28 ± 1.96^{a12}
a*	CNS	18.92 ± 1.22^{c1}	17.19 ± 1.63^{c1}	15.15 ± 1.58^{b1}	12.77 ± 1.74^{a1}
	CRS	19.06 ± 0.77^{c1}	17.81 ± 1.32^{bc1}	16.59 ± 1.81^{b12}	14.10 ± 3.54^{a1}
	RFNS	18.40 ± 1.69^{b1}	18.66 ± 1.20^{b1}	17.46 ± 0.75^{b2}	13.91 ± 1.41^{a1}
	RFRS	19.04 ± 1.45^{b1}	18.04 ± 1.90^{b1}	18.34 ± 1.27^{b2}	13.91 ± 1.74^{a1}
	LFNS	17.71 ± 0.48^{b1}	17.20 ± 3.22^{b1}	16.69 ± 0.85^{b12}	12.59 ± 0.75^{a1}
	LFRS	18.71 ± 1.19^{b1}	17.70 ± 0.90^{b1}	16.86 ± 1.20^{b12}	12.87 ± 0.76^{a1}
b*	CNS	19.57 ± 3.33^{a23}	20.25 ± 1.99^{a2}	19.43 ± 1.85^{a2}	19.46 ± 1.34^{a234}
	CRS	20.47 ± 1.35^{a3}	20.27 ± 1.33^{a2}	19.89 ± 2.36^{a2}	18.13 ± 2.75^{a23}
	RFNS	21.49 ± 2.37^{a3}	21.71 ± 1.37^{a2}	19.80 ± 1.70^{a2}	19.51 ± 1.32^{a34}
	RFRS	21.18 ± 2.33^{a3}	22.06 ± 2.40^{a2}	21.81 ± 1.64^{a2}	20.85 ± 0.98^{a4}
	LFNS	15.79 ± 1.10^{a1}	16.71 ± 1.73^{a1}	15.69 ± 2.07^{a1}	15.79 ± 1.56^{a1}
	LFRS	17.25 ± 1.67^{a12}	16.49 ± 2.68^{a1}	16.17 ± 1.18^{a1}	17.13 ± 1.02^{a12}

For sample denomination see Table 1. Means ± standard deviation. Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).

Ruiz-Capillas & Jiménez-Colmenero, 2010; Triki et al., 2013) but are lower than those reported by El Ayachi, Daoudi, and Benkerroum (2007).

During refrigerated storage, a slight significant increase ($P < 0.05$) in TVC and LAB levels was observed for all batches, with LAB the predominant flora. The increase observed was generally lower than observed in similar fresh products (Mathenjwa et al., 2012; Ruiz-Capillas, Cofrades, Serrano, & Jiménez-Colmenero, 2004; Triki et al., 2013) where after 3–5 days storage levels of 8 log cfu/g were reported. The low growth rate may be attributed mainly to the preservative effect of the sodium metabisulphite together with the low storage temperature (2 ± 1 °C). Mathenjwa et al. (2012) observed the same effect of the use of SO_2 in the formulation of S. African fresh sausages during refrigerated storage. Paleari-Bioanchi, Beretta, Cattaneo, and Balzaretti (1985) reported that *Staphylococcus aureus*, *Escherichia coli* and *Lactobacillus* spp. growth in vitro were inhibited by sulphite.

The effect of SO_2 was also observed on the enterobacteria where a significant decrease ($P < 0.05$) was noted during storage, reaching levels between 1.30 and 2.98 log cfu/g by day 10 of storage, mainly in the LFNS, LFRS and RFRS batches. In a previous study (Triki et al., 2013) on merguez sausages formulated without sodium metabisulphite, the enterobacteria levels were one unit (log) higher than observed in this study. Sodium metabisulphite is most active against Gram-negative microorganisms, particularly *Enterobacteriaceae* (Banks & Board, 1982). Sulphite is effective against microorganisms only when present in the free (unbound) form and it is most potent at low temperatures. The antimicrobial activity is the result of the undissociated sulphurous acid which enters the cell and reacts with thiol groups of proteins, enzymes and cofactors (Davidson, Sofos, & Branen, 2005).

Other authors have observed a decrease in the amount of all bacterial groups in the first three days of storage in merguez formulated with a commercial organic acid mixture (El Ayachi et al., 2007). Banks and Board (1982) showed sulphite-inhibition of

Table 7
Microbiological counts (log cfu/g) in the merguez sausages during the refrigerated storage.

Microorganisms	Samples	Storage time (days at 2 °C)			
		0	3	6	10
Total viable count	CNS	5.88 ± 0.11^{a2}	5.78 ± 0.00^{a3}	5.88 ± 0.07^{a3}	6.43 ± 0.05^{b2}
	CRS	5.37 ± 0.01^{a1}	5.39 ± 0.12^{a2}	5.52 ± 0.12^{b1}	6.10 ± 0.02^{c1}
	RFNS	5.33 ± 0.14^{a1}	5.20 ± 0.04^{a1}	5.51 ± 0.03^{b1}	6.45 ± 0.04^{c2}
	RFRS	5.37 ± 0.14^{a1}	5.19 ± 0.06^{a1}	5.52 ± 0.15^{b1}	6.69 ± 0.03^{c3}
	LFNS	5.56 ± 0.19^{a1}	5.27 ± 0.10^{a12}	5.68 ± 0.07^{b2}	6.80 ± 0.03^{c3}
	LFRS	5.44 ± 0.13^{a1}	5.22 ± 0.06^{a1}	5.56 ± 0.03^{b12}	6.64 ± 0.02^{c3}
Lactic acid bacteria	CNS	5.60 ± 0.01^{a2}	5.45 ± 0.21^{a4}	5.83 ± 0.04^{b3}	6.44 ± 0.03^{c123}
	CRS	5.24 ± 0.34^{a1}	5.15 ± 0.21^{a34}	5.23 ± 0.04^{a1}	6.13 ± 0.02^{b1}
	RFNS	4.89 ± 0.05^{a1}	4.50 ± 0.65^{a1}	5.33 ± 0.04^{b12}	6.38 ± 0.00^{c12}
	RFRS	4.90 ± 0.01^{a1}	4.60 ± 0.43^{a1}	5.28 ± 0.14^{b12}	6.53 ± 0.03^{c23}
	LFNS	5.06 ± 0.13^{a1}	4.69 ± 0.30^{a12}	5.59 ± 0.05^{b23}	6.77 ± 0.02^{c3}
	LFRS	4.95 ± 0.03^{a12}	5.00 ± 0.21^{a23}	5.37 ± 0.01^{b12}	6.61 ± 0.01^{c23}
Enterobacteriaceae	CNS	4.15 ± 0.21^{b3}	4.30 ± 0.00^{b3}	3.00 ± 0.00^{a2}	2.98 ± 0.31^{a4}
	CRS	3.56 ± 0.09^{c2}	3.39 ± 0.55^{c2}	3.00 ± 0.00^{b2}	2.56 ± 0.17^{a3}
	RFNS	3.62 ± 0.18^{c2}	3.30 ± 0.43^{bc12}	3.06 ± 0.08^{b2}	2.66 ± 0.11^{a34}
	RFRS	3.58 ± 0.33^{d2}	3.00 ± 0.00^{c1}	2.00 ± 0.71^{b1}	1.60 ± 0.00^{a12}
	LFNS	3.35 ± 0.04^{d12}	3.00 ± 0.00^{c1}	2.00 ± 0.71^{b1}	1.30 ± 0.43^{a1}
	LFRS	3.17 ± 0.08^{b1}	3.00 ± 0.00^{b1}	2.00 ± 0.71^{a1}	1.77 ± 0.10^{a2}

For sample denomination see Table 1. Means ± standard deviation. Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).

Enterobacteriaceae during the storage of British unripened fresh sausage.

Although NaCl has been described as antimicrobial no clear difference was observed between normal and reduced sodium samples. This may be due to a greater antimicrobial effect of the sulphite which masks the possible effect of the sodium.

3.9. Biogenic amines

Table 8 shows biogenic amine contents. Initially, phenylethylamine, tyramine, tryptamine and cadaverine were not detected and histamine and agmatine were lower than 1 mg/kg; only the physiological amines spermidine and espermine along with putrescine had higher initial levels, mainly spermine with levels of 14–17 mg/kg. Although significantly lower levels of spermine were reported in the batches containing konjac with oil (RFNS and RFRS), the values were close to the other samples. The initial amine levels were similar to those observed in other studies of merguez (Triki et al., 2013) and lower than those determined in restructured beef steak (Ruiz-Capillas et al., 2004) or *longaniza* type fresh sausages (Ruiz-Capillas & Jiménez-Colmenero, 2010).

During storage, the physiological amines showed few changes except for the spermine in the LFNS and LFRS batches where a significant decrease was seen at the end of storage, and for the spermidine in the control batches (CNS and CRS) which increased ($P < 0.05$). The decrease in spermine have been attributed to a de-amination reaction and/or microbial consumption (Bover-Cid, Hugas, Izquierdo-Pulido, & Vidal-Carou, 2001).

Phenylethylamine and putrescine clearly increased throughout storage; this was greater ($P < 0.05$) in the control batches (CNS and CRS) with values of 3.31–3.38 and 4.16–4.38 mg/kg respectively. The histamine levels at the end of storage were less than 0.2 mg/kg and agmatine levels less than 0.4 mg/kg. The reduction in the levels of these amines due to the effect of sulphites has also been reported (Bover-Cid et al., 2001). Other important biogenic amines in meat products in chilled storage, such as tyramine and cadaverine, were not detected. This, along with the generally low levels of biogenic amines found is due mainly to the low microorganism levels in these merguez sausages (Table 7). This behaviour was also observed in a study of longaniza type fresh sausage, where similar counts were also related to very low levels (Ruiz-Capillas & Jiménez-Colmenero, 2010). However, in a previous study of merguez during refrigerated storage and reformulated without SO_2 the amine levels were much higher, mainly tyramine and histamine, and microorganism counts were also higher.

Overall, no clear effect ($P > 0.05$) of replacing NaCl was observed on the production of biogenic amines, a result which agrees with its imperceptible influence on microbial growth (Table 7). It is worth noting the clear relationship which exists between the low formation of biogenic amines and presence of metabisulphite as preservative. Nevertheless, it should be taken into account that sodium sulphite may encourage the presence of specific type of flora, which in some cases may give rise to greater production of a specific biogenic amine, as occurred in this study, where the presence of sulphites was seen to have slightly stimulated the production of phenethylamine and putrescine (Table 8). This has also been observed by others (Bozkurt & Erkmen, 2002; Ruiz-Capillas & Jiménez-Colmenero, 2010). Bover-Cid et al. (2001) also observed lower microbial counts and levels of cadaverine in sausages produced with different levels of sulphite compared with control samples. In contrast, tyramine and putrescine production seemed to be stimulated by the presence of sodium sulphite, these authors observed a clear effect of sulphite on the accumulation of putrescine and other biogenic amines. However the microbiological spoilage and production of biogenic amines in a fermented product such as that studied by Bover-Cid et al. (2001) is very different from that in a fresh product, as described in this paper.

4. Conclusion

The merguez reformulation strategy based on the use of konjac gel and olive oil stabilized in a konjac matrix as fat replacer, and on partial sodium chloride substitution by potassium, calcium and magnesium

Table 8
Biogenic amine levels (mg/kg) in the merguez sausages during the refrigerated storage.

Biogenic amines	Samples	Storage time (days at 2 °C)			
		0	3	6	10
Phenylethylamine	CNS	ND	1.10 ± 0.05^{a3}	2.01 ± 0.00^{b3}	3.38 ± 0.08^{c5}
	CRS	ND	1.28 ± 0.06^{a4}	2.42 ± 0.12^{b4}	3.31 ± 0.09^{c5}
	RFNS	ND	0.76 ± 0.01^{a2}	1.27 ± 0.03^{b1}	1.62 ± 0.37^{c1}
	RFRS	ND	1.37 ± 0.13^{a4}	1.98 ± 0.03^{b3}	2.16 ± 0.08^{c3}
	LFNS	ND	1.28 ± 0.24^{a4}	2.10 ± 0.09^{b3}	2.25 ± 0.01^{c4}
	LFRS	ND	0.64 ± 0.04^{a1}	1.46 ± 0.02^{b2}	2.02 ± 0.01^{c2}
Putrescine	CNS	1.97 ± 0.04^{a3}	2.92 ± 0.09^{b3}	3.16 ± 0.03^{c4}	4.38 ± 0.24^{d3}
	CRS	0.88 ± 0.03^{a1}	2.71 ± 0.05^{b3}	2.95 ± 0.16^{b34}	4.16 ± 0.01^{c3}
	RFNS	1.84 ± 0.02^{a3}	2.32 ± 0.01^{b2}	2.56 ± 0.04^{b1}	3.30 ± 0.06^{c2}
	RFRS	1.73 ± 0.18^{a23}	2.32 ± 0.02^{b2}	2.65 ± 0.06^{c12}	3.42 ± 0.32^{d2}
	LFNS	1.60 ± 0.05^{a2}	2.35 ± 0.01^{b2}	2.66 ± 0.04^{c12}	3.44 ± 0.04^{d2}
	LFRS	1.75 ± 0.05^{a23}	2.05 ± 0.03^{b1}	2.86 ± 0.04^{c23}	2.99 ± 0.01^{c1}
Spermidine	CNS	3.07 ± 0.34^{a2}	3.22 ± 0.09^{a4}	2.99 ± 0.11^{a2}	4.90 ± 0.03^{b3}
	CRS	3.17 ± 0.23^{a2}	3.02 ± 0.10^{a34}	2.77 ± 0.14^{a2}	4.20 ± 0.22^{b2}
	RFNS	2.23 ± 0.78^{a1}	2.60 ± 0.03^{a123}	2.19 ± 0.09^{a1}	3.78 ± 0.02^{b2}
	RFRS	2.37 ± 0.30^{ab1}	2.62 ± 0.26^{b23}	1.98 ± 0.27^{a1}	2.83 ± 0.08^{b1}
	LFNS	2.23 ± 0.25^{a1}	2.46 ± 0.16^{a12}	2.71 ± 0.32^{a2}	2.68 ± 0.11^{a1}
	LFRS	2.66 ± 0.05^{b12}	2.06 ± 0.02^{a1}	2.55 ± 0.01^{ab2}	2.81 ± 0.19^{b1}
Spermine	CNS	16.72 ± 0.51^{a2}	16.14 ± 0.88^{a3}	15.81 ± 0.83^{a3}	16.26 ± 0.63^{a3}
	CRS	16.98 ± 0.28^{c2}	15.96 ± 0.38^{ab23}	15.42 ± 0.65^{a23}	16.43 ± 0.46^{bc3}
	RFNS	14.67 ± 0.07^{a1}	16.24 ± 0.09^{bc3}	15.38 ± 0.29^{ab23}	16.39 ± 0.50^{c3}
	RFRS	15.12 ± 0.13^{b1}	15.10 ± 0.24^{b12}	14.68 ± 0.09^{ab12}	13.82 ± 0.47^{a2}
	LFNS	16.24 ± 0.43^{c2}	15.40 ± 0.32^{c23}	14.36 ± 0.05^{b1}	12.08 ± 0.05^{a1}
	LFRS	16.80 ± 0.59^{c2}	14.41 ± 0.06^{b1}	14.58 ± 0.25^{b12}	12.15 ± 0.25^{a1}

For sample denomination see Table 1. Means ± standard deviation. Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).
ND: Not detected.

salts with the addition of sodium metabisulphite as a preservative, permitted a reduction of fat by 32–80% and of sodium by 36–40%. The shelf life was greatly improved in the reformulated merguez, up to 10 days compared to the control samples through the addition of the preservative during chilled storage. The reformulate sausages also had satisfactory sensory and technological properties. Therefore this processing strategy is suitable and recommended for use in the development of healthier fresh merguez sausages, from the point of view of fat, salt and mineral levels.

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V. DISCUSIÓN INTEGRADORA

V. DISCUSIÓN INTEGRADORA

La industria cárnica, al igual que otros sectores de la alimentación, está experimentando importantes transformaciones como consecuencia de innovaciones tecnológicas y cambios en las demandas de los consumidores. Una de las principales tendencias que marca la evolución del consumo de derivados cárnicos surge de la preocupación de los consumidores por la salud, favorecida por las nuevas recomendaciones y orientaciones nutricionales impulsadas por diversas instituciones (Organización Mundial de la Salud, Ministerio de Sanidad y Consumo, etc.). De este modo se está incrementado el consumo de productos percibidos como más "saludables", los cuales para su desarrollo requieren procesos de reformulación encaminados a potenciar la presencia de compuestos beneficiosos, y/o limitar la de aquellos otros con efectos negativos.

En este sentido, la grasa es uno de los constituyentes de los alimentos a los que se ha prestado mayor atención debido a que es un factor que, a través de diversos mecanismos, condiciona en mayor o menor medida la aparición de diversos problemas de salud como enfermedades coronarias (arteriosclerosis, trombosis, etc.), obesidad, cáncer, etc. En España en torno al 35% de la grasa ingerida diariamente (126 g) es de origen cárnico (Varela et al., 1996). Es por ello que una de las principales metas en relación con la salud radica en mejorar el contenido lipídico (reducir la proporción de grasa y aproximar su perfil de ácidos grasos a recomendaciones de salud) (NAOS, 2005). Por otro lado, el consumo de niveles elevados de sal (sodio) está directamente relacionado con un aumento de la hipertensión arterial que favorece la incidencia de enfermedades cardiovasculares. Dado que en España el consumo de sodio (9.8 g/día) es muy superior al recomendado (5 g/día) (NAOS, 2009) y que aproximadamente el 26% del sodio ingerido procede del consumo de derivados cárnicos, resulta esencial plantear estrategias de reducción de sodio en estos alimentos.

Recientemente se ha firmado un convenido entre la Agencia del Ministerio de Sanidad, AESAN (2012) con la Confederación Española de Detallistas de la Carne (CEDECARNE) y la Asociación de Fabricantes y Comercializadores de Aditivos y Complementos Alimentarios (AFCA) para **reducir las cantidades de sal y grasa en los productos de carnicería-charcutería** de elaboración tradicional, concediendo un plazo

de dos años para que los productos mencionados tengan un 10% menos de sal y un 5% menos de grasa (AESAN, 2012), como indica el lema "menos grasa y sal más salud"

En este contexto cobra especial relevancia el desarrollo de estrategias de reformulación de productos cárnicos para lograr estos objetivos, manteniendo similares atributos de calidad (sensoriales, seguridad, conveniencia, etc.) que los productos tradicionales. De este modo, el consumo de productos reformulados más saludables podrían satisfacer las expectativas de los consumidores.

Sin embargo, se debe tener en cuenta que las estrategias, encaminadas a producir modificaciones en la composición, además de requerir cambios de reformulación, también pueden requerir de modificaciones en los procesos de elaboración y conservación. Todo ello además, de influir en las propiedades tecnológicas, sensoriales y microbiológicas de los productos, puede condicionar la formación de algunos compuestos potencialmente tóxicos para la salud, como por ejemplo las aminas biógenas (Figura V.1).

Las aminas biógenas pueden causar migrañas, dolores de cabeza, problemas gástricos e intestinales, y respuestas pseudo-alérgicas, principalmente debidas a la acción tóxica de histamina y tiramina (apartado I.1.3.1). Además, algunos de estos compuestos (tiramina, putrescina y cadaverina) han sido señalados como precursores de nitrosaminas, compuestos potencialmente cancerígenos, presentando además interés desde un punto de vista más tecnológico por su empleo como índices de calidad en distintos productos sometidos a diferentes tratamientos.

Por todo lo expuesto el objetivo fundamental planteado en esta memoria ha consistido en **desarrollar procesos de reformulación de derivados cárnicos encaminados a obtener productos más saludables y estudiar como dichos procesos condicionan la formación de aminas biógenas**. Este estudio se ha realizado en dos tipos de productos cárnicos típicos y muy apreciados por los consumidores de distintos países, y con distintas características y condiciones de procesado: chorizo (producto crudo curado) y merguez (producto fresco). Los procesos de reformulación planteados están dirigidos a incidir en el contenido lipídico (reduciendo la presencia de grasa y mejorando su perfil de ácidos grasos) y/o limitar la presencia de sodio.

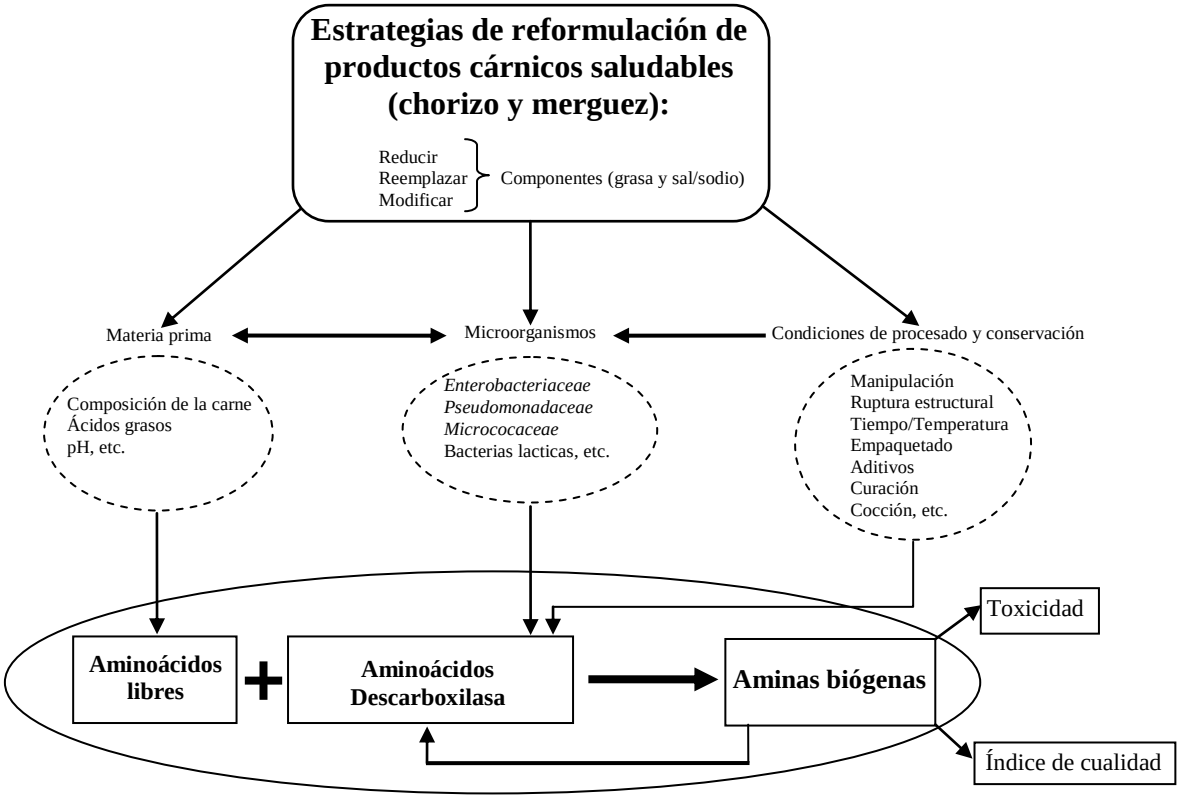

Figura V.1. Factores asociados a la reformulación de productos cárnicos (chorizo y merguez) que pueden afectar la formación de aminas biógenas

V.1. MEJORA DEL PROCEDIMIENTO DE DETERMINACIÓN DE AMINAS BIOGENAS EN PRODUCTOS CÁRNICOS

En una primera fase de esta memoria se ha planteado **desarrollar un procedimiento mejorado para la determinación de aminas biógenas en productos cárnicos** por HPLC **(capítulo IV.1.1)**. Se consideró imprescindible disponer de un método adecuado de determinación simultánea de las distintas aminas biógenas habitualmente presentes en los productos cárnicos que se iban a estudiar. Como punto de partida se empleó un método de determinación cromatográfico que presentaba la ventaja de tener una alta sensibilidad y buena resolución, además de ser sencillo y rápido (Ruiz-Capillas & Moral, 2001). La determinación se realiza en una columna de intercambio catiónico acoplada a un sistema post-columna empleando el o-ophtalaldehido (OPA) como derivatizante y usando un detector de fluorescencia. Sin

embargo, este método, optimizado para muestras de pescado, permitía únicamente determinar 7 aminas biógenas (tiramina, histamina, putrescina, cadaverina, agmatina, espermidina y espermina) (Figura V.2), siendo necesario su adaptación y validación para matrices cárnicas. Además, se consideró conveniente cuantificar adicionalmente dos nuevas aminas biógenas la β-feniletilamina y la triptamina, dado su presencia relevante en carne y productos cárnicos, así como por su interés toxicológico.

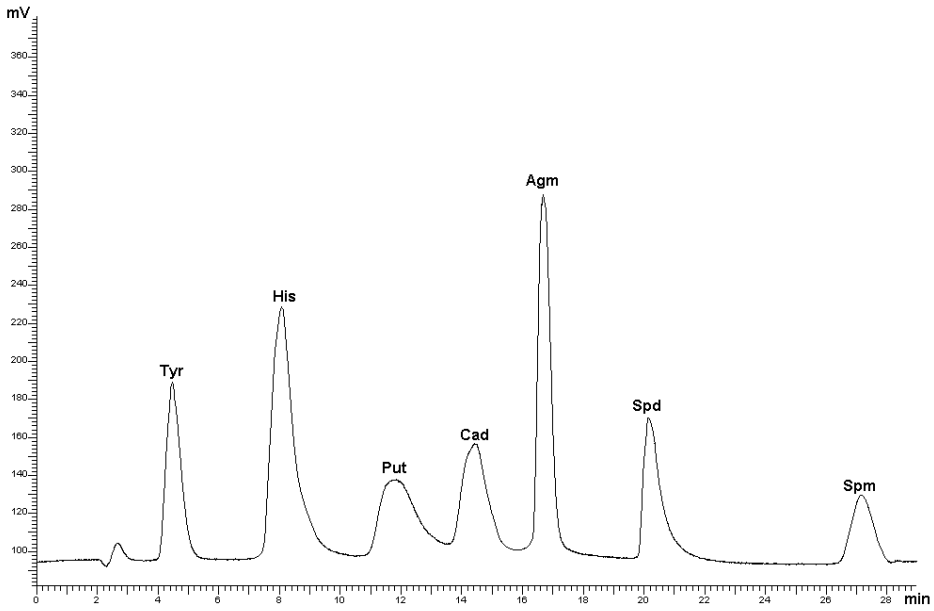

Figura V.2. Cromatograma de un patron de 4 mg/L de 7 aminas biógenas con el método original de Ruiz-Capillas & Moral (2001)

Para llevar a cabo este objetivo, primeramente se realizó una optimización del método **(capítulo IV.1.1)**, empleando inicialmente un patrón conteniendo además las dos nuevas aminas biógenas. El cromatograma obtenido mostró que la resolución de β-feniletilamina y triptamina no era adecuada (Figura V.3). Para mejorar dicha resolución se realizaron cambios en los distintos parámetros claves en la etapa de separación cromatográfica como fueron la temperatura de la columna y del coil de reacción, la velocidad del flujo, composición y pH de las fases móviles, etc. Como era de esperar, y puesto que la determinación se realiza en una columna de intercambio catiónico, los cambios tanto en el pH de las fases móviles (fase A: pH= 6,33; fase B: pH= 5,63, fase C: pH= 13,00) como en el flujo de la bomba (con el óptimo en 0,8 ml/min) fueron los parámetros más significativos en la puesta a punto del método. Esto permitió una

adecuada resolución de todas las aminas biógenas y una reducción muy notable del tiempo de análisis (Figura V.4).

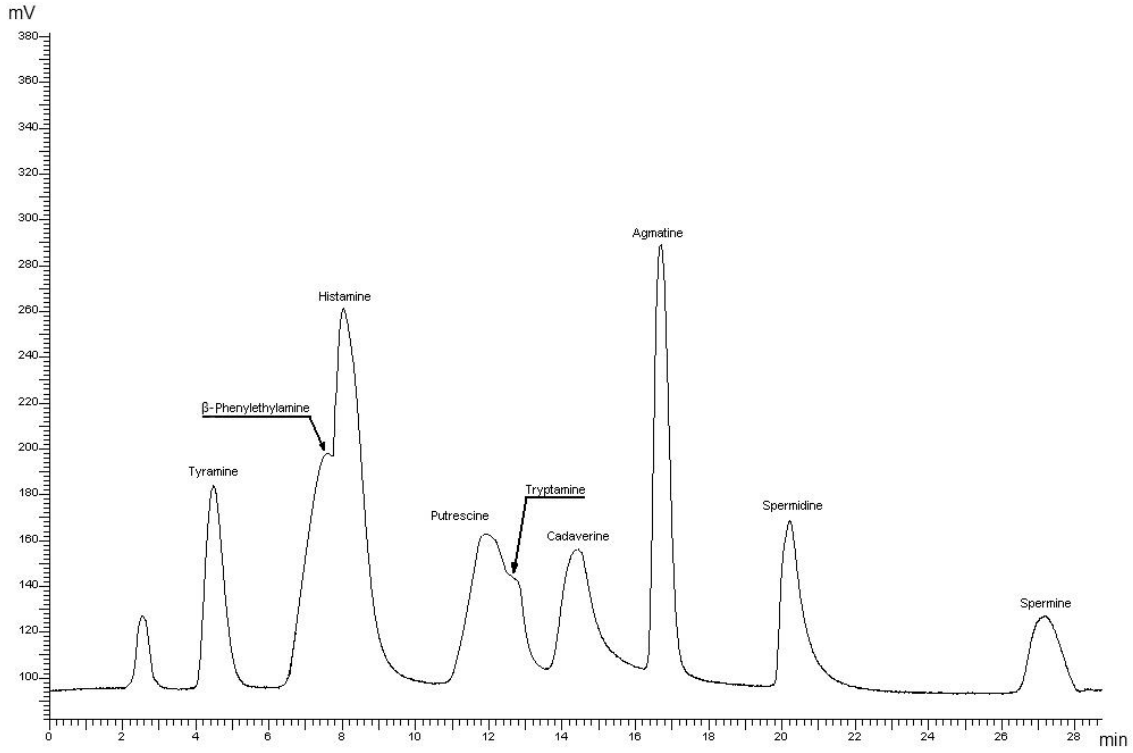

Figura V.3. Cromatograma de un patron de 4 mg/L de las nueve aminas biógenas con el método original de Ruiz-Capillas & Moral (2001)

Una vez optimizado el método se procedió a su validación **(capítulo IV.1.1)** empleando criterios de linealidad, sensibilidad, precisión y repetibilidad. El método optimizado presentó un coeficiente de regresión (R^2) > 0,99 para la linealidad. Los límites de detección y cuantificación fueron de 0,03 a 0,10 mg/L y de 0,10 a 0,20 mg/L, respectivamente. La precisión de los tiempos de retención presentaron una desviación estándar menor de 0,07 (excepto para la triptamina: 0,19) (Figura V.4).

Se estudió la recuperación en la fase de extracción de las aminas biógenas en distintos extractos cárnicos procedentes de carne fresca, salchichas frankfurt y chorizo y se observó que estaba por encima de 98% **(capítulo VI.1.1**; Figura V.5**)**.

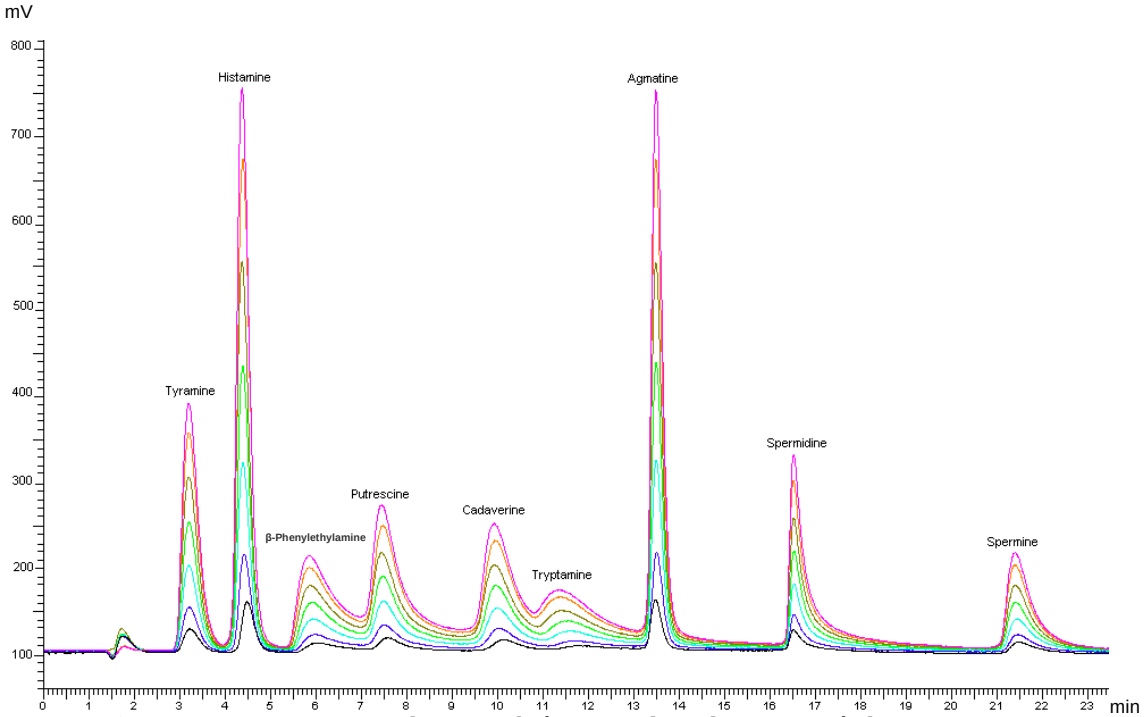

Figura V.4. Cromatograma de aminas biógenas en las soluciones estándar 0,05-12 mg/L

El método optimizado resultó adecuado para la determinación de las aminas biógenas en un amplio rango de concentración en los productos cárnicos ensayados, los cuales presentaban distinta composición y condiciones de procesado (Figura V.5), demostrando así la versatilidad del método **(capítulo IV.1.1)**.

Una vez optimizado el método de determinación de aminas biógenas en productos cárnicos, se procedió al **ensayo de procesos de reformulación encaminados a mejorar el contenido lipídico y/o limitar la presencia de sodio en dos tipos de productos cárnicos:** chorizo (ejemplo de producto crudo curado) **(capítulo IV.2.1, capítulo IV.2.2, capítulo IV.2.3 y capítulo IV.2.4)** y merguez (ejemplo de producto fresco) **(capítulo IV.3.1 y capítulo IV.3.2)** (Figura V.1). En ambos casos además de valorar las consecuencias del cambio de composición sobre las propiedades físico-químicas, sensoriales y microbiológicas de los productos, se ha estudiado **su efecto sobre la formación (cantidad y perfil) de aminas biógenas**. Las distintas características de los productos estudiados aconsejan plantear su discusión de manera individualizada.

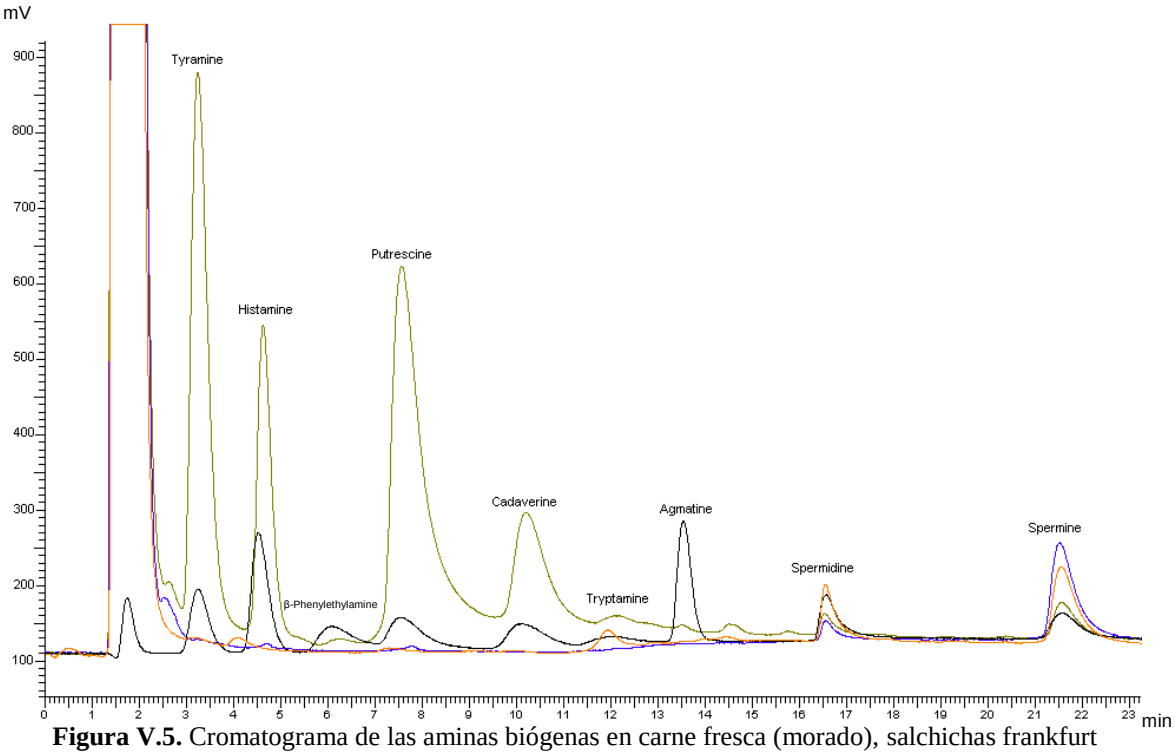

Figura V.5. Cromatograma de las aminas biógenas en carne fresca (morado), salchichas frankfurt (naranja), chorizo (verde) y solución estándar de 4 mg/L (negro).

V.2. EFECTO DE LOS PROCESOS DE REFORMULACIÓN DEL CHORIZO EN LA FORMACIÓN DE AMINAS BIÓGENAS

Como estrategia de **reformulación del chorizo** se planteó la sustitución de grasa animal (tocino de cerdo) por un sustituto de grasa constituido por un gel de konjac glucomanano (KG). Este glucomanano presenta interesantes propiedades tecnológicas con potenciales beneficiosos para la salud. Se eligió el chorizo por ser un producto típico en España y en otros países mediterráneos, muy consumido y que contiene elevadas proporciones de grasa (> 30%). Se estudiaron distintos niveles de sustitución de grasa animal (0, 50, 80%) por la misma proporción de KG **(capítulo IV.2.1 y capítulo IV.2.2)**. En base a los resultados y al sistema de procesado establecidos en este primer estudio se abordó un segundo experimento en el que se pretendía simultáneamente reducir los niveles de grasa de los productos (75% de sustitución de la grasa por KG en el lote LFKG) y mejorar el perfil de ácidos grasos de éstos **(capítulo IV.2.3 y capítulo IV.2.4)**. Para este segundo estudio se empleó como sustituto de la grasa animal, una matriz de konjac con aceite retenido en su interior (KGM) (90 y 100% de sustitución de la grasa por KGM en las muestras LFK10 y LFK20, respectivamente).

El material lipídico elegido fue una combinación de aceites elegido para dotar al derivado cárnico de un perfil lipídico más saludable. Tal combinación estaba constituida por aceites de origen vegetal (oliva y lino) y de origen animal (pescado), originando una mezcla que presenta una pequeña proporción de AGS, gran cantidad de AGMI y AGPI (incluyendo gran cantidad de AGPI *n-3* de cadena larga) y adecuado balance de n-6/n-3 y AGPI/AGS (Delgado-Pando et al., 2010a). Se realizó un estudio de conservación en refrigeración de los chorizos reformulados.

A nivel de composición los procesos de reformulación tuvieron varias consecuencias. Generalmente los productos presentaron proporciones más elevadas de humedad y carbohidratos por la adición del konjac **(Tabla 1, capítulo IV.2.1 y Tabla 2, capítulo IV.2.3)**. Pero el efecto más significativo en la composición de los elaborados se puso de manifiesto en relación con el componente lipídico. Por un lado, se alcanzó una importante reducción en los niveles de grasa, con valores comprendidos entre el 34 y el 69% respecto al control, asociados a una notable disminución del contenido energético, entre 14,8 y 46% **(Tabla 1, capítulo IV.2.1 y Tabla 2, capítulo IV.2.3)**. Dada la mayor proporción de grasa **(capítulo IV.2.3)**, la muestra control presentó el mayor contenido en ácidos grasos AGS y AGMI, siendo muy similar la presencia de AGPI en los productos reformulados con la matriz de konjac elaborado con la mezcla de aceites (KGM) (Figura V.6-a), **(capítulo IV.2.3)**. Además, el proceso de reformulación ha sido determinante para condicionar el perfil lipídico de los productos. Mientras que la reducción de grasa no produjo cambios en la proporción de ácidos grasos (Figura V.6-b), como era de esperar en los productos formulados sólo con grasa de cerdo (muestras NF versus LFKG), en aquellos otros en los que se sustituyo grasa animal por la combinación de aceites, se produjo una importante mejora en el perfil lipídico (LFK10 y LFK20) (Figura V.6-b). Tal mejora se reflejó en una importante disminución de AGS junto con un notable incremento en los niveles de AGPI (especialmente n-3), asociado además con un aumento (de 2 a 3 veces con respecto al control) de la relación AGPI/AGS (Figura V.6-c).

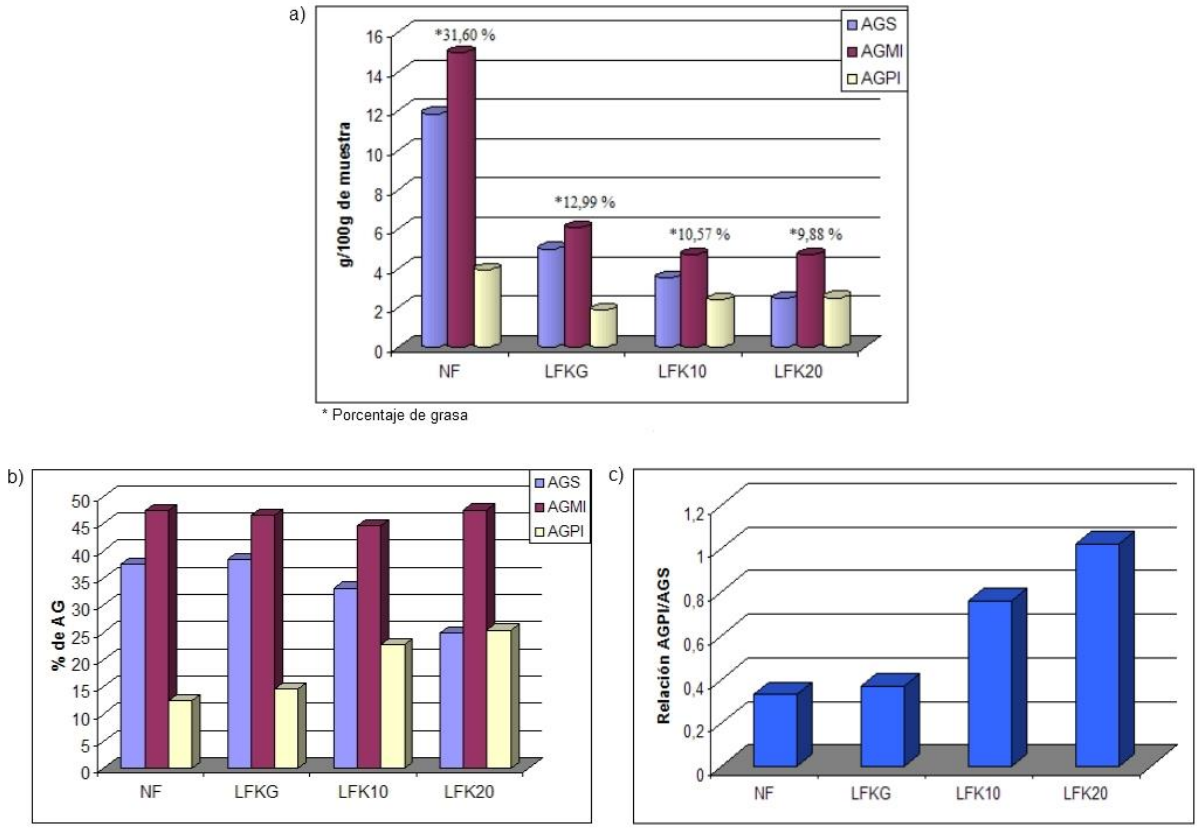

Figura V.6. Composición lipídica del chorizo: **a)** Contenido en ácidos grasos (g/100g) (**capítulo IV.2.3**); **b)** Porcentajes de ácidos grasos (**capítulo IV.2.3**); **c)** Relación AGPI/AGS (**capítulo IV.2.3**).
Lotes: NF: contenido normal de grasa animal (18,5%); LFKG: sustitución al 75% por KG; LFK10: sustitución al 90% por KGM elaborado con 10% de mezcla de aceites; LFK20: sustitución al 100% por KGM elaborado con 20% de mezcla de aceites

Teniendo en cuenta la citada composición lipídica **(capítulo IV.2.3 y capítulo IV.2.4)**, y de acuerdo con el reglamento (CE) 1924/2006, a los productos reformulados se le pueden atribuir varias declaraciones nutricionales. Así por ejemplo al chorizo reformulado sustituyendo el 90% de la grasa animal por el konjac conteniendo 10% de la mezcla de aceites (LFK10), responde a las declaraciones recogidas en la Tabla V.1. De igual modo y tomando en consideración el reglamento (CE) nº 423/2012 que recoge las declaraciones de propiedades saludables autorizadas, ese mismo producto puede presentar las declaraciones reflejadas en la Tabla V.2. Todo ello abre importantes expectativas para el consumidor con los consiguientes beneficios de comercialización.

Tabla V.1. Declaraciones de propiedades nutricionales aplicables al chorizo reformulado sustituyendo el 90% de la grasa animal por el konjac conteniendo 10% de la mezcla de aceites (LFK10) **(capítulos IV.2.3 y IV.2.4)**. Adaptado del reglamento (CE) 1924/2006.

Declaraciones	**Condiciones generales de uso**	**Valores relacionados con las condiciones de uso**
Valor energético reducido	Sólo puede usarse si el valor energético del alimento se reduce, como mínimo, en un 30% con una indicación de la característica o características que provocan la reducción del valor energético total del alimento.	Reducción del 46%
Fuente de proteínas	Sólo puede usarse con alimentos fuentes de proteínas que aportan como mínimo el 12% del valor energético del alimento	Aporta el 44,9% del valor energético
Alto contenido de proteínas	Sólo puede usarse con alimentos fuentes de proteínas que aportan como mínimo el 20% del valor energético del alimento	Aporta el 44,9% del valor energético
Fuente de ácidos grasos omega 3	Sólo puede usarse con alimentos que contienen al menos 0,3 g de ácido alfa-linolénico por 100 g y por 100 kcal o al menos 40 mg de la suma de ácido eicosapentaenoico (EPA) y ácido decosahexaenoico (DHA) por 100 g y por 100 kcal.	Contiene 0,87 g de alfa-linolénico/ 100 g Contiene 160 mg de EPA + DHA / 100 g
Alto contenido de ácidos grasos omega 3	Sólo puede usarse con alimentos que contienen al menos 0,6 g de ácido alfa-linolénico por 100 g y por 100 kcal o al menos 80 mg de la suma de ácido eicosapentaenoico y ácido decosahexaenoico por 100 g y por 100 kcal.	Contiene 0,87 g / 100 g de alfa-linolénico Contiene 160 mg de EPA + DHA / 100 g

Además de cambios de composición, los procesos de reformulación llevan aparejados cambios en las propiedades tecnológicas, sensoriales y microbiológicas de los productos. El impacto sobre los parámetros de color fue limitado, siendo sólo significativo cuando se empleaban niveles elevados de konjac (LF) en sustitución de la grasa animal **(Tabla 2, capítulo IV.2.1)**. Este hecho se atribuyó principalmente a que el empleo de las especias, colorantes y saborizantes para la elaboración de este tipo de productos, minimizaba el efecto de la sustitución de grasa. Sin embargo, los mayores cambios provocados por la reformulación fueron observados a nivel de textura, estando condicionados por el tipo de sustituto de grasa. En tal sentido se observó que los chorizos reformulados con gel de konjac sin aceite (KG) presentaban mayor ($P<0.05$) dureza y masticabilidad, estando correlacionados ambos parámetros con las perdidas de peso (0,880; $P<0,001$). Por contra, la sustitución de grasa animal por la matriz de konjac conteniendo la combinación de aceites originó productos más blandos, con parámetros de textura más parecidos a los del chorizo control **(Figura 1, capítulo IV.2.3)**.

Tabla V.2. Declaraciones de propiedades saludables aplicables al chorizo reformulado sustituyendo el 90% de la grasa animal por el konjac conteniendo 10% de la mezcla de aceites (LFK10) **(capítulos IV.2.3 y IV.2.4)**. Adaptado al reglamento (CE) Nº 432/2012.

Compuestos	Declaraciones	Condiciones generales de uso	Valores relacionados con las condiciones de uso
Ácido alfa-linolénico:	El ácido alfa-linolénico contribuye a mantener niveles normales de colesterol sanguíneo.	Sólo puede usarse para los alimentos considerados como FUENTE DE ÁCIDOS GRASOS OMEGA-3 del Reglamento (CE) no 1924/2006. Se informará al consumidor de que el efecto beneficioso se obtiene con una ingesta diaria de 2 g de este ácido graso.	Contiene 0,87 g de alfa-linolénico/100 g
Ácido docosahexaenoico	Contribuye a mantener el funcionamiento normal del cerebro y al mantenimiento de la visión en condiciones normales.	Sólo puede usarse con alimentos que contienen un mínimo de 40 mg de ácido docosahexaenoico por 100 g y por 100 Kcal. Para que un producto pueda llevar esta declaración, se informará al consumidor de que el efecto beneficioso se obtiene con una ingesta diaria de 250 mg de ácido docosahexaenoico.	Contiene 61,48 mg de DHA/100 g
EPA/DHA	Los ácidos eicosapentaenoico (EPA) y docosahexaenoico (DHA) contribuyen al funcionamiento normal del corazón.	Sólo puede usarse con alimentos que son, como mínimo, fuente de EPA y DHA de acuerdo con la declaración FUENTE DE ÁCIDOS GRASOS OMEGA-3del Reglamento (CE) no 1924/2006. Para que un producto pueda llevar esta declaración, se informará al consumidor de que el efecto beneficioso se obtiene con una ingesta diaria de 250 mg de EPA y DHA.	Contiene 160 mg de EPA + DHA /100 g
Proteína	Aumento y conservación de la masa muscular y mantenimiento de los huesos en condiciones normales.	Sólo puede usarse respecto a alimentos que son, como mínimo, fuente de proteínas de acuerdo con la declaración FUENTE DE PROTEÍNAS del Reglamento (CE) no 1924/2006.	Aporta el 44,9% del valor energético

Estos cambios se vieron reflejados en la valoración sensorial de los chorizos que, en aquellos formulados con gel de konjac, fueron bien aceptados por el panel de catadores, no presentando diferencias significativas a nivel de apariencia y sabor frente al control **(Tabla 2, capítulo IV.2.1)**. Sin embargo, en aquellos productos elaborados con matrices de konjac conteniendo mezcla de aceites se produjo una disminución en su apreciación sensorial, especialmente en relación con los atributos de dureza y sabor **(Figura 1, capítulo IV.2.3)**, exhibiendo generalmente los productos reformulados peor puntuación en jugosidad que en el control. En todo caso, de entre los derivados cárnicos con presencia de aceite, el de menor contenido (LFK10), presentaba los mejores niveles de aceptabilidad **(Tabla 4, capítulo IV.2.3)**.

Los procesos de reformulación ensayados no condicionaron la microbiota de los productos, siendo los cambios producidos durante el procesado los característicos de este tipo de elaborados cárnicos. En tal sentido, durante las fases de secado y

maduración se produjo un aumento significativo del crecimiento microbiano, principalmente bacterias aerobias viables totales (TVC) y de bacterias ácido-lácticas (LAB), llegando a niveles superiores a 8 Log cfu/g al final del proceso, y siendo las bacterias ácido-lácticas la flora dominante en todos los casos **(Tabla 1, capítulo IV.2.2).** Las modificaciones de composición tampoco condicionaron cambios microbiológicos de los productos a lo largo del almacenamiento **(Tabla 1, capítulo IV.2.2 y Tabla 4, capítulo IV.2.4)**, donde se observó que los niveles de microorganismos (TVC y LAB) se mantuvieron elevados y constantes tanto en los productos control como en los reformulados con KG y KGM, sin diferencias significativas entre ellos **(Tabla 1, capítulo IV.2.2 y Tabla 4, capítulo IV.2.4)**.

Un aspecto esencial de este estudio radica en valorar como los cambios de composición inducidos en los productos, asociados a su procesado, pueden condicionar la cantidad y tipo de aminas biógenas. Los resultados obtenidos indican que la formación de aminas biógenas (en especial las de origen principalmente microbiano), se vieron afectadas de forma distinta por el proceso de reformulación, variando con el tipo de amina. Las aminas fisiológicas (espermidina y espermina) apenas se vieron afectadas, observandose niveles elevados en el producto desde el inicio. Aminas como triptamina y histamina, también presentaron cantidades muy bajas durante el procesado **(Tabla 2, capítulo IV.2.2)**. Sin embargo, el proceso de reformulación afectó a las demás aminas biógenas. Su variación estuvo condicionada por un lado por los niveles de sustitutos de grasa empleados (50%, 75%, 80%, 90% y 100%), y por otro por el tipo de análogo usado (gel de konjac o gel de konjac con aceite: KG o KGM, respectivamente). En la primera fase de procesado de los chorizos se apreció un aumento significativo de los niveles de las aminas biógenas más representativas de la carne (tiramina y putrescina), que siguieron aumentando hasta el final del procesado. Se observó que el incremento de los niveles de tiramina estaba en función del nivel de sustitución de la grasa animal por el KG **(Tabla 2, capítulo IV.2.2)** y KGM **(Tabla 5, capítulo IV.2.4)**. Esta relación se ha apreciado también en los valores de putrescina, pero sólo en el caso de las muestras elaboradas con KG **(Tabla 2, capítulo IV.2.2)** y no en las formuladas con KGM **(Tabla 5, capítulo IV.2.4)**.

En el caso de la cadaverina, amina característica en este tipo de productos crudos curados, se observaron niveles más elevados en las muestras elaboradas con KGM **(Tabla 5, capítulo IV.2.4)** que en los reformulados con KG **(Tabla 2, capítulo IV.2.2)**. En general la formación de aminas biógenas en estos productos cárnicos está asociada al

crecimiento microbiano, principalmente de bacterias ácido-lácticas (Ruiz-Capillas & Jimenez-Colmero, 2004). Sin embargo, en estos estudios, el aumento de aminas biógenas estaba algo retrasado en el tiempo en relación con el experimentado a nivel microbiológico **(Tabla 1 y 2, capítulo IV.2.2 y Tabla 4 y 5, capítulo IV.2.4)**. En tal sentido, el efecto del crecimiento microbiano se vió reflejado en la produccion de aminas biógenas un tiempo después de que la población microbiana alcanzase niveles superiores a 8 Log cfu/g. Este hecho podría ser debido a que los microorganismos, principales productores de aminas biógenas, necesitan un tiempo para sintetizar cantidades suficientes de las enzimas aminoácido descarboxilasas responsables de la producción de aminas biógenas. Además, los cambios observados en los niveles de aminas biógenas en funcion de la reformulacion, no se correspondia con los valores totales de los microorganismos estudiados. En tal sentido se podría sugerir la presencia de cepas específicas en las que la producción de aminas biógenas pueda variar selectivamente en función de la naturaleza del sustrato (la carne, la grasa animal y tipo de sustituto de grasa). Tal hipótesis abre nuevas vías de investigación futura.

Además, de durante el procesado, la influencia de la reformulación en la producción de aminas biógenas fue valorada en condiciones de conservación en estado refrigerado. Como cabía esperar también se observó que durante el almacenamiento de estos productos se producía en general un aumento en el contenido de las aminas biógenas **(Tabla 5, capítulo IV.2.4)**. Dicho aumento dependía de la composición (reformulación) de los productos, variando tanto con el tipo de sustituto empleado, como con los niveles de sustitución de grasa realizados. Al final del período de conservación las aminas biógenas presentes en mayor concentración fueron tiramina y cadaverina (en las muestras reformuladas con KG y con menores niveles de KGM), seguidas de espermina y putrescina que presentaron los niveles más elevados en los chorizos elaborados con menores y mayores niveles de aceite adicionados, respectivamente. Como ocurría durante el procesado, estas concentraciones de aminas biógenas están asociadas con niveles elevados de microorganismos principalmente de bacterias ácido lácticas, aunque todos los productos presentaron valores superiores a 8 Log cfu/g. De nuevo cabe señalar que las diferencias en las concentraciones de aminas biógenas entre distintas formulaciones podrían estar asociadas a las especificidades de cepas.

En general no se aprecia un efecto claro en los niveles de aminas biógenas en función de las estrategias de reformulación empleadas

V.3. EFECTO DE LOS PROCESOS DE REFORMULACIÓN DEL MERGUEZ EN LA FORMACIÓN DE AMINAS BIÓGENAS

Teniendo en cuenta los resultados derivados del estudio de chorizo, se abordó el proceso de **reformulación de un producto fresco como el merguez**. Dicho producto fue seleccionado por su importancia en los países del Magreb (Norte de África, su origen de producción), así como en algunos países europeos con una representación notable de esas comunidades. Además, supone ampliar la gama de productos cárnicos más saludables a alimentos "étnicos" para los que existe una escasa oferta a pesar de ser un sector con destacadas perspectivas de futuro. Se consideró interesante mejorar la composición de estos productos como modelo de procesado en fresco y elevado contenido en grasa y sal (sodio). A pesar de la importancia que tiene tal planteamiento no se conocen estudios que incidan en su desarrollo.

En una primera fase **(capítulo IV.3.1)** se abordaron los procesos de reducción y mejora del contenido lipídico. Para tal fin se realizó la sustitución de grasa animal (vacuno) por los análogos ya ensayados en el chorizo, esto es gel de konjac (KG) (75/KG y 100/KG) y matriz de konjac con aceite de oliva incorporado (OKM) (75/OKM y 100/OKM); el criterio de elección del aceite fue establecida en base a dos aspectos: su contenido en AGMI (ácido oleico) y disponibilidad en los países en los que generalmente elaboran y consumen el merguez. Sin embargo, a pesar de los beneficios alcanzados a nivel lipídico con el proceso de reformulación realizado, el producto presentaba aún dos serías limitaciones, contenía elevados niveles de sodio y su estabilidad era muy reducida. Por tal motivo, en una segunda fase del estudio se ensayó, por un lado una estrategia de reducción de sodio basada en la sustitución del cloruro sódico empleado en su formulación por una mezcla de sales (KCl, $CaCl_2$ y $MgCl_2$). Por otro lado la adición de un conservante de tipo sulfito (metabisulfito de sodio), en los niveles marcados por la legislación (0,045%) a fin de aumentar la vida útil de estos productos.

Si se analiza el efecto de los procesos de reformulación a nivel de composición se puede señalar lo siguiente. Como ocurria en el caso del chorizo, la magnitud de los cambios están asociados al tipo de análogo de grasa empleado y su nivel de sustitución **(capítulo IV.3.1 y capítulo IV.3.2)**. Así, los productos refromulados con KG fueron los que contenian niveles de humedad mayores junto a menores niveles de proteína **(Tabla**

2, capítulo IV.3.1 y Tabla 2, capítulo IV.3.2). Especialmente significativos fueron los cambios en el contenido en grasa, donde se observó una reducción entre el 33 y el 80%, porcentajes de reducción con respecto al control muy similares a los encontrados en el chorizo **(capítulo IV.2.1 y capítulo IV.2.3)**.

Dada la mayor proporción de grasa, la muestra control presentó el mayor contenido en ácidos grasos (AGS, AGMI), siendo muy similar la presencia de AGPI con los productos reformulados con la matriz de konjac y aceite (OKM) (Figura V.7-a) **(capítulo IV.3.1 y capítulo IV.3.2)**, como ocurría en el chorizo. Sin embargo, un análisis cualitativo del perfil lipídico permite señalar que, comparados con el control (contenido habitual de grasa animal), los productos reformulados presentaban menores proporciones de AGS y mayores de AGMI y AGPI (Figura V.7-b), sobre todo en aquellos que contenían la matriz de konjac con aceite de oliva (OKM). En tal caso se duplicó (75/OKM / RFNS) e incluso triplicó (100/OKM) el valor de la relación AGPI/AGS con respecto al control (Figura V.7-c) **(capítulos IV.3.1 y capítulo IV.3.2)**. Todo ello acompañado con notable descenso en el contenido energético (23-58%), dependiendo del tipo de sustitución de grasa animal empleado **(Tabla 2, capítulo IV.3.1 y Tabla 2, capítulo IV.3.2)**.

A nivel de composición, la reformulación del merguez originó una importante reducción de sodio (del 36 al 40%), así como un aumento de otros minerales como K, Ca y Mg. Tales resultados se derivaron de la estrategia de sustitución del 50% del NaCl, por una mezcla de sales compuesta por KCl, $CaCl_2$ y $MgCl_2$. En tales condiciones, estos productos contribuirían a la reducción de la ingesta de sodio (Desmond, 2006; NAOS, 2009, Directiva 2008/100/CE). Además supondrían una importante fuente de otros minerales, al aportar un 10-15% de la ingesta diaria recomendada (Directiva 2008/100/CE) en potasio, 8-10% en calcio y 10-20% en magnesio **(capítulo IV.3.2)**.

Teniendo en cuenta estos resultados y de acuerdo con el reglamento (CE) 1924/2006, a varios de los productos reformulados se les pueden atribuir diversas declaraciones nutricionales. Así por ejemplo, las correspondientes al merguez reformulado con contenido reducido en grasa (sustituyendo el 75% de grasa de vacuno por la matriz de konjac con aceite de oliva) y sal, se recogen en la Tabla V.3. De igual modo y tomando en consideración el reglamento (CE) nº 423/2012 que recoge las declaraciones de propiedades saludables autorizadas, ese mismo producto puede presentar las declaraciones reflejadas en la Tabla V.4. Todo ello, como en el caso del

chorizo, abre importantes expectativas para el consumidor con los consiguientes beneficios de comercialización.

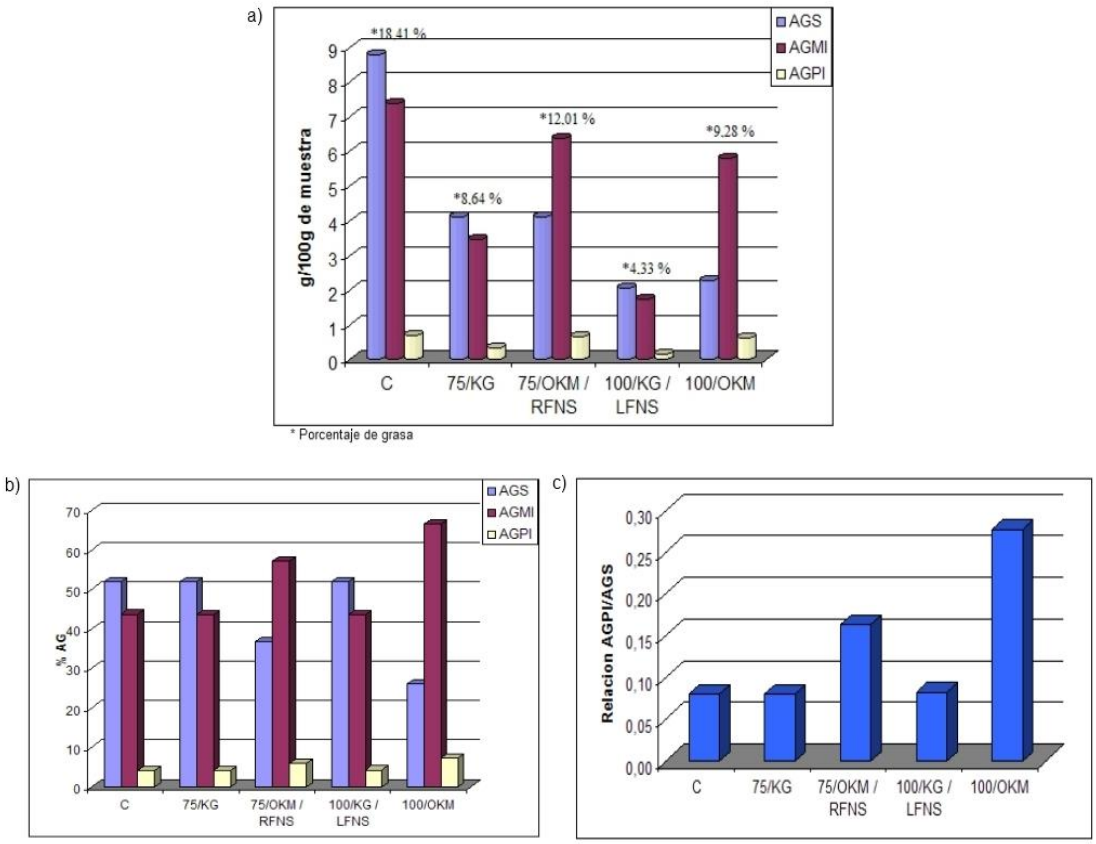

Figura V.7. Composición lipídica del merguez **a)** Contenido en ácidos grasos (g/100g) (**capítulos IV.3.1, IV.3.2); b)** Porcentajes de ácidos grasos (**capítulos IV.3.1, IV.3.2); c)** Relación AGPI/AGS (**capítulos IV.3.1, IV.3.2)**

Lotes: C: contenido normal de grasa animal (29%); 75/KG: sustitución al 75% por KG; 75/OKM / RFNS: sustitución al 75% por OKM; 100/KG / LFNS: sustitución al 100% por KG; 100/OKM: sustitución al 100% por OKM

Además de cambios de composición, los procesos de reformulación del merguez llevaban aparejados cambios en las propiedades tecnológicas, sensoriales, y microbiológicas de los productos. En tal sentido se observó como la sustitución de grasa animal por análogos a base de konjac originó cambios en la naturaleza de la matriz cárnica, formando estructuras más blandas. Sin embargo, el efecto de la reducción de sal o del empleo del antimicrobiano no se vió reflejado en los parámetros de textura **(Tabla 4, capítulo IV.3.2)**.

Tabla V.3. Declaraciones de propiedades nutricionales aplicables al merguez reducido en grasa y sal y con perfil lipídico mejorado elaborado sustituyendo el 75% de la grasa de vacuno por una matriz de konjac y aceite de oliva (RFRS) **(capítulo IV.3.2)**. Adaptado al reglamento (CE) 1924/2006.

Declaraciones	**Condiciones generales de uso**	**Valores relacionados con las condiciones de uso**
Fuente de proteínas	Sólo puede usarse con alimentos fuentes de proteínas que aportan como mínimo el 12% del valor energético del alimento	Aporta 34,77% del valor energético
Alto contenido de proteínas	Sólo puede usarse con alimentos fuentes de proteínas que aportan como mínimo el 20% del valor energético del alimento	Aporta 34,77% del valor energético
Alto contenido de ácidos grasos monoinsaturados.	Sólo puede usarse si al menos 45% de los ácidos grasos presentes en el producto proceden de grasas monoinsaturadas y las grasas monoinsaturadas aportan más del 20% del valor energético del producto.	Contiene 59,94% de los ácidos grasos Contiene 35,06 % del valor energético
Contenido reducido en sodio	Sólo puede usarse si el valor de sodio del alimento se reduce, como mínimo, en un 25%	Reducción del 40% del valor de sodio
Fuente de hierro	Sólo puede usarse con alimentos fuentes de hierro que aportan como mínimo el 15% del la CDR (14 mg).	Aporta 16,64% de la CDR

Por otro lado, los cambios en composición de los productos también se vieron reflejados en las propiedades ligantes de grasa y agua por cuanto aumentaban las perdidas por cocción. En tal sentido se observó una alta correlación de 0,810 ($P<0.01$) entre las pérdidas por cocción y la cantidad de grasa de los productos **(Tabla 4, capítulo IV.3.1 y Tabla 3, capítulo IV.3.2)**. Además, se apreció un claro efecto de la reducción de sal en las pérdidas de agua durante la cocción **(Tabla 3, capítulo IV.3.2)**, de manera que los productos que contenían menos proporción de sal presentaron mayores pérdidas.

La sustitución de la grasa animal por KG y OKM en el merguez originó una disminución en los parámetros de color L* y b* pero no afectó al parámetro a* **(Tabla 6, capítulo IV.3.1)**. Sin embargo, se observó que la adición de SO_2 no tuvo efecto en los parámetros L* y b* en todos los lotes excepto en el producto con bajo contenido en grasa, independientemente del contenido en sodio. En general se observó que los parámetros de color estaban más influenciados por el tipo de grasa o análogo y su contenido, que por las distintas sales empleadas en la reformulación **(Tabla 5, capítulo IV.3.2)**.

Tabla V.4. Declaraciones de propiedades saludables aplicables al merguez reducido en grasa y sal y con perfil lipídico mejorado elaborado sustituyendo el 75% de la grasa de vacuno por una matriz de konjac y aceite de oliva (RFRS) **(capítulo IV.3.2)**. Adaptado al reglamento (CE) Nº 432/2012.

Componentes	Declaraciones	Condiciones generales de uso	Valores relacionados con las condiciones de uso
Ácidos grasos monoinsaturados	Mantenimiento de niveles normales de colesterol sanguíneo.	Sólo puede usarse respecto a alimentos con alto contenido de ácidos grasos insaturados, de acuerdo con la declaración ALTO CONTENIDO DE GRASAS INSATURADAS que figura en el anexo del Reglamento (CE) no 1924/2006.	Contiene 5,58 g de ácidos grasos monoinsaturados / 100 g
Proteína	Aumento y conservación de la masa muscular y mantenimiento de los huesos en condiciones normales.	Sólo puede usarse respecto a alimentos que son, como mínimo, fuente de proteínas de acuerdo con la declaración FUENTE DE PROTEÍNAS del Reglamento (CE) no 1924/2006.	Aporta 34,77% del valor energético
Alimentos con un contenido bajo o reducido en sodio	Un menor consumo de sodio contribuye a mantener la tensión arterial normal.	Sólo puede usarse respecto a alimentos que contienen, como mínimo, un nivel bajo de sodio o sal, de acuerdo con la declaración BAJO CONTENIDO DE SODIO/SAL o un nivel reducido de sodio o sal, de acuerdo con la declaración CONTENIDO REDUCIDO DE [NOMBRE DEL NUTRIENTE] que figuran en el anexo del Reglamento (CE) no 1924/2006.	Reducción del 40% del valor de sodio
Hierro	El hierro contribuye a la función cognitiva normal, al metabolismo energético normal, a la formación normal de glóbulos rojos y de hemoglobina, al transporte normal de oxígeno en el cuerpo, al funcionamiento normal del sistema inmunitario, al proceso de división celular y ayuda a disminuir el cansancio y la fatiga.	Sólo puede usarse respecto a alimentos que son, como mínimo, fuente de hierro de acuerdo con la declaración FUENTE DE [NOMBRE DE LAS VITAMINAS] Y/O [NOMBRE DE LOS MINERALES] que figura en el anexo del Reglamento (CE) no 1924/2006.	Aporta 16,64% de la CDR.

A pesar de que el proceso de reformulación del merguez condicionó diversos parámetros tecnológicos y de composición (grasa, sal, etc.) y varios de ellos con gran incidencia en su apreciación organoléptica, el panel de catadores consideró que todas las formulaciones presentaron una valoración adecuada a nivel de jugosidad, dureza y aceptabilidad general. El uso de SO_2 no tuvo tampoco ninguna repercusión negativa en términos sensoriales **(capítulo IV.3.2)**. Estos resultados se han relacionado, como en el caso del chorizo, con el tipo de producto que incorpora gran cantidad de especias en su elaboración, minimizando las consecuencias originadas por efecto de los cambios de composición.

Como en el caso del chorizo, las estrategias de sustitución de grasa animal por konjac en la reformulación de un producto fresco (merguez) no afectaron al crecimiento microbiano: aerobios viables totales (TVC), bacterias acido-lácticas (LAB) o enterobacterias **(Tabla 7, capítulo IV.3.1)**. Sin embargo, como cabía esperar, el empleo

del SO_2 provocó un descenso significativo del crecimiento microbiano a lo largo de la conservación en refrigeración, y particularmente en los niveles de bacterias ácido-lácticas **(Tabla 6, capítulo IV.3.2)**. Esto supuso un aumento de vida útil desde los 3 (en ausencia de SO_2) hasta los 10 días. Las consecuencias de la presencia de SO_2 sobre el desarrollo microbiano también se vieron reflejadas en el perfil de aminas biógenas. Así en los productos elaborados con SO_2 **(Tabla 7, capítulo IV.3.2)**, se apreció una menor formación de aminas biógenas en comparación con los mismos productos elaborados sin SO_2 **(Tabla 8, capítulo IV.3.1)**. Esto sería consecuencia de la acción inhibidora del conservante que limita la presencia de microorganismos formadores de aminas biógenas. Esta mínima formación de aminas biógenas no permitiría apreciar el efecto de la estrategia de reducción de sodio sobre su producción **(Tabla 7, capítulo IV.3.2)**.

Además de a nivel cuantitativo, la presencia de SO_2 originó cambios cualitativos en el perfil de aminas biógenas. Así por ejemplo, las aminas biógenas más afectadas por el empleo del metabisulfito de sodio fueron tiramina, histamina y cadaverina, con un importante descenso en sus niveles, llegando incluso a no detectarse tiramina y cadaverina durante el almacenamiento en refrigeración **(Tabla 7, capítulo IV.3.2)**. Sin embargo, los niveles de β-feniletilamina, putrescina y espermidina aumentaron en los productos con SO_2, especialmente en el merguez control frente a los reformulados. Esto podría ser atribuido a un fenómeno de selección microbiana generado por la adición del sulfito que habría favorecido selectivamente la proliferacion de cepas microbianas y productoras de β-feniletilamina y putrescina. Este comportamiento ha sido descrito por otros autores en los productos cárnicos en presencia de diferentes aditivos (Bozkurt & Erkmen, 2002; Ruiz-Capillas & Jiménez-Colmenero, 2010).

En lo que respecta a las aminas fisiológicas, espermidina y espermina presentaron los niveles más altos al inicio del almacenamiento, con algunas variaciones a lo largo de la conservación sin relación aparente con los factores de composición o procesado **(Tabla 8, capítulo IV.3.1 y Tabla 7, capítulo IV.3.2)**. Los niveles de aminas fisiológicas fueron menores en las muestras de merguez con konjac (KG y OKM) en presencia de SO_2 **(Tabla 7, capítulo IV.3.2)**.

En el caso de las aminas de deterioro, sin el conservante adicionado, putrescina y cadaverina presentaron concentraciones muy bajas con independencia del proceso de reformulación **(Tabla 8 capítulo IV.3.1 y Tabla 7 del capítulo IV.3.2)**. Sin embargo, se observó un incremento muy significativo en los niveles de tiramina e histamina a lo largo de la conservación en los productos elaborados sin el conservante **(Tabla 8**

capítulo IV.3.1). Sólo en el caso de la histamina, se pudo relacionar su contenido con el empleo de los sustitutos de grasa, apreciándose mayor formación de histamina en los productos con el contenido lipídico mejorado.

A excepcion de las aminas fisiológicas, la formación de aminas biógenas en estos transformados cárnicos ha sido muy diferente en comparacion con los observados en los estudios de chorizo. Este hecho parece lógico teniendo en cuenta que se trata de elaborados muy diferentes. En relación con productos de naturaleza análoga también se aprecian algunas diferencias en el contenido y perfil de aminas biógenas. Tales diferencias (en espermina, histamina y tiramina) cabe atribuirlas principalmente a los ingredientes (tanto cárnicos, como no cárnicos) empleados en la elaboración de este producto, algunos de los cuales condicionan el crecimiento de una flora microbiana específica (Figura V.1). Este es el caso de, además del SO_2, de otros ingredientes como la harissa, pimentón, cilandro, hinojo, etc., a los que se les han atribuido propiedades antimicrobianas (Tajkarimi et al. 2010), pudiendo condicionar la microbiota en estos productos así como su capacidad aminoácido descarboxilasa.

A pesar de estos estudios, los niveles de aminas biógenas encontrados tanto en el chorizo como en el caso del merguez estaban por debajo de los niveles que pueden suponer un factor de riesgo para la salud humana.

VI. CONCLUSIONES

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El desarrollo del trabajo contenido en esta memoria ha permitido obtener las siguientes conclusiones:

1.- La metodología de determinación de aminas biógenas desarrollada permite la separación, identificación y cuantificación de nueve aminas biógenas (tiramina, histamina, β-feniletilamina, putrescina, cadaverina, triptamina, agmatina, espermidina y espermina) en diferentes matrices cárnicas y condiciones de procesado. Las principales ventajas que reporta en relación al método de partida, se manifiestan a nivel de versatilidad, sensibilidad y tiempo de ejecución.

2.- La sustitución de grasa animal por un gel de konjac o por una matriz de konjac conteniendo una combinación de aceites (de origen vegetal y marino) resultó una estrategia adecuada para la obtención de un producto cárnico crudo-curado tipo chorizo potencialmente funcional en base a un contenido lipídico mejorado. Este planteamiento permite, por un lado, reducciones importantes de grasa (34-69%) y por otro, dotar al producto de proporciones elevadas de ácidos grasos (0,87 g de alfa-linolénico y 60 mg de EPA + DHA en 100 g). Dicha estrategia si bien presenta algunas implicaciones en las propiedades tecnológicas y sensoriales, permite la obtención de productos con niveles convenientes de aceptabilidad sensorial.

3.- La estrategia de reformulación ensayada para la obtención de chorizo con un perfil lipídico más saludable no condiciona el tipo y evolución de la microbiota, más influida por efecto del procesado que por los cambios de composición. Sin embargo, dicha estrategia afectó la formación de algunas aminas biógenas (tiramina, cadaverina putrescina o espermidina). Dado que no se observó una relación evidente entre los niveles de aminas biógenas y la carga microbiana, se ha sugerido la existencia de cepas específicas en las que la producción de aminas biógenas pueda variar selectivamente en función de la naturaleza del sustrato.

4.- La sustitución de grasa animal (vacuno) por un gel de konjac o por una matriz de konjac conteniendo aceite de oliva resultó una estrategia adecuada para la obtención de

una salchicha fresca tipo merguez con un contenido lipídico mejorado. Dicha estrategia permite reducciones importantes de grasa (33-80%), favorece además la presencia del aceite de oliva, que si bien conlleva modificaciones en las características de los productos, estos presentaron adecuadas propiedades tecnológicas y atributos sensoriales.

5.- Procesos de reformulación de merguez basados en la combinación de estrategias que abarcan el empleo de análogos de grasa (reemplazando la grasa de vacuno), la sustitución de NaCl por una combinación de otras sales y la utilización de un antimicrobiano (SO_2), permitieron la obtención de salchicha frescas tipo merguez potencialmente funcionales y de prolongada vida útil. Tales estrategias, si bien produjeron una mejora del perfil lipídico y una reducción del contenido en sodio (36-40%), condicionaron las propiedades tecnológicas, aunque los productos obtenidos presentaron adecuados niveles de apreciación sensorial.

6.- En salchichas tipo merguez, mientras que las modificaciones en el contenido y perfil lipídico no condicionaron la microbiota, la presencia de SO_2 originó una disminución acusada de la carga microbiana (independientemente de la reducción de sodio), responsable del aumento de la vida útil de este producto. Sin embargo, la producción de aminas biógenas estaba condicionada por los niveles de sustitución de grasa y el tipo de sustituto empleado, aunque solamente en el caso de ausencia de conservante. El empleo del SO_2 causó una reducción muy acusada de los niveles de aminas biógenas asociado al tipo de amina biógena.

Como **conclusión general** hay que señalar que a través de la estrategia de reformulación planteada a lo largo de esta memoria, se pueden elaborar productos cárnicos saludables, de contenido en grasa y/o sodio reducido, estables, seguros, con propiedades tecnológicas y organolépticas adecuadas y con un perfil lipídico mejorado de acuerdo a recomendaciones nutricionales (menor cantidad de ácidos grasos saturados y mayor de poliinsaturados). Todo ello, supone que estos productos, chorizo y meguez, son susceptibles de acogerse a varias de las declaraciones nutricionales y de propiedades saludables de los alimentos.

VII. REFERENCIAS

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